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CALCIUM CHLORIDE AND ALCOHOL

EFFECT OF THERAPEUTIC DOSES OF CALCIUM CHLORIDE ON THE CLINICAL STAGE OF ALCOHOLIC INTOXICATION AND ON BLOOD ALCOHOL[1]

By

A. R. ALHA

(Received for publication May 27, 1950)

Graham (4) observed in 1828 that calcium chloride forms molecular compounds with alcohols. Later on the structure of the molecular compound formed by anhydrous calcium chloride and ethyl alcohol was expressed by such formulae as $CaCl_2 \cdot 3C_2H_5OH$ (5) and $2CaCl_2 \cdot 7C_2H_5OH$ (9). But no compound formed by this molecular compound and water, with a constant water content, has so far been found.

This quality of calcium chloride was soon made use of industrially to isolate alcohol, and to determine it quantitatively in mixtures. Ant' Vuorinen (1) described a method based on it by which he established the alcohol content of liqueurs and essences.

Knowing this chemical quality of calcium chloride we may ask ourselves whether it has any importance in living organism. The purpose of the present study is to find out whether calcium chloride, when given in therapeutic doses, has any effect on the blood alcohol content and on intoxication at a clinical stage, produced by test drinks and, on the other hand, whether alcohol affects the serum calcium values.

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[1] This study was assisted by a grant from the Temperance and Alcohol Research Committee of the Ministry of Social Affairs.

It is well known that calcium also regulates the functioning of the nervous system, and it has been used in the treatment of states of nervous over-excitement (2, 10).

The present study was carried out in connection with a larger series of studies on the relation of blood alcohol and the clinical stage of intoxication. A group of 22 prisoners in the Central Prison at Helsinki volunteered as test subjects. From among them three were chosen as showing expressive clinical symptoms of intoxication in experiments in which alcohol was given in the ratio of 1 g per kg of body weight. Before the investigation, which began in January this year, the test subjects had taken no alcohol for several months.

Test subject No 1, a chauffeur aged 24, was 168.5 cm tall and weighed 66—67 kg in the course of the experiments. The subject was of athletic build, of usual bone structure, scanty fatty tissue, and schizoid by psychic habitus. He had taken regularly large amounts of alcohol and also narcotics previous to imprisonment some years ago.

Test subject No 2, a smelter aged 24, 182 cm tall, weighing 80.5 kg. His body was pyknic, bone structure usual, fatty tissue moderate, psychic habitus cyclothyme. Previous to imprisonment, about 1½ years ago, he had taken large amounts of alcohol periodically.

Test subject No 3, an electric works operator aged 23, 173 cm tall, weighing 66.5—67.5 kg, of leptosomatic build, slender framework, scanty fatty tissue, schizothymic by psychic habitus. Before imprisonment about a year ago he had taken alcohol in moderation.

The experiments were performed in the mornings at about one week's intervals, starting usually at 8—9 a.m., and the test subjects came to the experiments after fasting 12 hours. During the experiment they were allowed to smoke freely.

In tests carried out on rabbits (2), when calcium compounds were given by the mouth in great quantities, a clear increase could be observed in the serum calcium, and a maximum in about 1—2 hours after the intake, and after about 6—8 hours the serum calcium had been restored to normal. But when the calcium compounds were given intravenously, the maximum occurred, as is natural immediately after the injection. The serum calcium decreased then very rapidly and after about 2 hours it differed very slightly from the control. This is a proof of the rapid elimination of calcium chloride from the blood, its diffusion into the tissue fluids, and of its simultaneous excretion into the urine or into the bowels.

The maximum concentration of the blood alcohol, on the other hand, occurs about ½—1½ hours after the end of the intake, if the alcohol has been taken in a comparatively short time (11).

In the present study these facts were applied with the view of dosing the calcium chloride so that the maxima of the serum calcium and of blood alcohol would occur simultaneously.

The maximum peroral dose of anhydrous calcium chloride is about 1 g (8). The maximum dose for intravenous injections is usually said to be 1 g (10 ml 10% solution). In the experiments 2 g $CaCl_2$ sicc. in 10 per cent solution (Kaisaniemi Pharmacy, Helsinki) was given per os and 10 ml 10 per cent solution (Orion) injected intravenously in 5 minutes. In one preliminary experiment Subject 1 was given 20 ml. Before the intake of calcium chloride or alcohol a blood sample was taken in all experiments to control the serum calcium and blood alcohol, and then blood samples during the whole experiment, usually after ½, 1, 2 and 3 hours from the beginning of the experiment.

The serum calcium was determined according to the modified Kramer-Tisdall (6) method as the mean value of two parallel determinations. In the literature the error of the method is considered to be $\pm$ 5 per cent (7). In the preliminary determinations the limit values usually deviated from each other by about 4 per cent. In one series of determinations, for instance, the following 6 values were obtained:

Mg per cent Ca: 9.12, 8.74, 9.03, 8.74, 8.93, 9.12.

A measured amount of 30.6 per cent alcohol solution mixed with water, 1 g per kg of body weight, was given in all the alcohol experiments. The test subjects ingested the alcohol in a very short time, practically in one draught. The samples for the blood alcohol were taken with a syringe needle from the vein into tubes prepared with oxalate and fluoride, and two Widmark's capillaries were filled out of each tube by slanting the tube. The alcohol determination was made according to Widmark's micromethod (11). The results of the parallel determinations, of which the mean value was taken, did not differ more than 0.05 per mil from each other in any of the cases.

Immediately after the blood samples a clinical examination of the stage of intoxication was performed according to the suggestion of the Blood Alcohol Committee (12) (see Table 3).

The pulse was counted within ½ min. on a subject standing. The smell of alcohol was of course not expressed quantitatively. Symptoms describing motor and psychological functions were expressed, as in the examination carried out by the Blood Alcohol Committee, in relative figures as follows:

0 = sure performance
1 = slightly uncertain performance
2 = uncertain performance
3 = wavering or completely uncertain performance.

Since any comparison between the degrees of these functions must be very subjective, the numerical value of certain types of performance was defined beforehand.

The numerical value 0 represents a sure performance. Yet it cannot be called normal, as many persons can show more or less uncertainty even physiologically, for instance in the walking test, in the Romberg test, and above all in the subtractio ntest. Moreover, even the same person may show slight daily deviations depending on his state of mind, due to sleepless night etc. A control examination was therefore carried out in all alcohol experiments before the taking of alcohol to find out the normal functions of the subjects.

The colour of the face was defined as follows: ± = pale, + = «usual», ++ = congestion, +++ = strong congestion.

DETAILS OF THE EXPERIMENTS

Experiment 1. — Physiological serum calcium at various times.

Experiment 2. — Serum calcium, blood alcohol and the clinical symptoms of intoxication when alcohol was ingested, at various times.

Experiment 3. — Serum calcium when calcium chloride was given per os, at various times.

Experiment 4. — Alcohol one hour after calcium chloride had been given per os. Serum calcium, blood alcohol, and the clinical symptoms of intoxication, at various times.

Experiment 5. — As in experiment 4, except that alcohol was given immediately after the intake of calcium chloride.

Experiment 6. — Calcium chloride intravenously. The first serum calcium determination 20 minutes after completion of injection; the others after 1, 2 and 3 hours.

Experiment 7. — First alcohol, then calcium chloride intravenously after 35—40 minutes. Serum calcium determinations as in previous experiment, blood alcohol and the clinical examination as in experiments 2, 4 and 5.

RESULTS AND CONCLUSIONS

Table 1 shows the serum calcium determinations, Table 2 the blood alcohol determinations and Table 3 the clinical examinations of the stage of intoxication. Table 3 shows also the clinical control examinations which were performed at the beginning of all the alcohol experiments, combined into one set of statistics for each subject, as they did not differ from one another (except for a slight difference in the pulse frequency — the average value of which appears in the Table).

I. SERUM CALCIUM

1. Experiment 1 gave the following limit values of the determinations made at various times:

Test subject	1:	8.74	and	9.41	mg	per cent
» »	2:	9.50	and	10.11	»	» »
» »	3:	9.89	and	10.27	»	» »

Test subject 1 and 2 showed some physiological change in the serum calcium at various times during the same morning, as the limit values deviated from each other more than $\pm$ 5% considered to be the common error in this method.

2. In the control tests made at the beginning of all experiments the following limit values were obtained:

Test subject	1:	8.37	and	9.02	mg	per cent
» »	2:	8.65	and	9.50	»	» »
» »	3:	9.02	and	10.27	»	» »

This shows that daily changes occurred in the serum calcium.

The existence of these changes makes it difficult to draw any conclusions about the possible changes of serum calcium in calcium chloride-alcohol experiments.

3. In experiments 3, 4 and 5 an evident though slight increase of a similar type was observed in the serum calcium of all test sub-

TABLE 1

SERUM CALCIUM AT VARIOUS TIMES, EXPRESSED IN MG PER CENT

Experiment	Subject N:o 1						
	8.00	9.00	9.20	9.30	10.00	11.00	12.00
Exp. 1. Physiological serum calcium		8.74		8.74	9.31	9.22	9.41
» 2. Alcohol at 9 o'clock		8.37		8.74	8.56	8.83	8.65
» 3. $CaCl_2$ per os at 9		9.01		9.37	9.65	9.83	9.65
» 4. $CaCl_2$ per os at 8 and alcohol at 9	8.65			9.58	9.58	9.67	9.58
» 5. $CaCl_2$ per os at 9 and alcohol directly afterwards		8.46		9.20	9.38	9.29	9.94
» 6. $CaCl_2$ intravenously at 9		9.02	11.35		10.70	10.42	
» 7. Alcohol at 8.20 and $CaCl_2$ intravenously at 8.55—9.00	8.84		10.23		9.67	9.58	9.11

Experiment	Subject N:o 2						
	8.00	9.00	9.20	9.30	10.00	11.00	12.00
Exp. 1. Physiological serum calcium		9.50		10.11	9.30	9.75	9.65
» 2. Alcohol at 9 o'clock		9.11		8.83	8.92	9.38	9.02
» 3. $CaCl_2$ per os at 9		8.65		8.92	9.46	9.65	9.92
» 4. $CaCl_2$ per os at 8 and alcohol at 9	8.93			9.39	9.39	9.67	9.58
» 5. $CaCl_2$ per os at 9 and alcohol directly afterwards		9.02		9.11	9.57	9.57	9.75
» 6. $CaCl_2$ intravenously at 9		8.93	9.67		9.49	9.58	
» 7. Alcohol at 8.20 and $CaCl_2$ intravenously at 8.55—9.00	9.11		9.95		9.86	9.77	9.77

Experiment	Subject N:o 3						
	8.00	9.00	9.20	9.30	10.00	11.00	12.00
Exp. 1. Physiological serum calcium		10.26		9.89	9.93	10.18	9.98
» 2. Alcohol at 9 o'clock		9.29		9.48	9.48	9.48	9.57
» 3. $CaCl_2$ per os at 9		9.19		9.37	9.56	9.46	9.74
» 4. $CaCl_2$ per os at 8 and alcohol at 9	9.49			10.23	10.14	10.23	9.77
» 5. $CaCl_2$ per os at 9 and alcohol directly afterwards		9.29		10.03	10.12	9.57	9.48
» 6. $CaCl_2$ intravenously at 9		9.02	10.14		9.77	9.49	
» 7. Alcohol at 8.20 and $CaCl_2$ intravenously at 8.55—9.00	9.86		10.97		11.25	10.97	10.04

TABLE 2

BLOOD ALCOHOL, EXPRESSED PER MIL. ½, 1, 2 AND 3 HOURS AFTER THE INTAKE OF ALCOHOL. CONTR. = BEFORE INTAKE

Experiment	Subject N:o 1					Subject N:o 2					Subject N:o 3				
	Contr.	½	1	2	3	Contr.	½	1	2	3	Contr.	½	1	2	3
Exp. 2. Alcohol at 9 o'clock	0.03	0.75	0.98	1.09	0.95	0.00	1.07	1.22	1.19	1.06	0.00	0.37	0.66	1.03	0.99
» 4. $CaCl_2$ per os at 8 and alcohol at 9		0.54	1.07	1.19	1.06		0.31	1.07	1.13	1.07		0.38	0.57	1.05	0.96
» 5. $CaCl_2$ per os at 9 and alcohol directly afterwards		0.80	1.31	1.15	1.04		0.50	0.82	1.14	1.03		0.30	0.64	1.13	1.09
» 7. Alcohol at 8.20 and $CaCl_2$ intravenously at 8.55—9.00		1.03	1.29	1.19	1.08		0.92	1.24	1.21	1.02		0.42	0.77	1.16	0.99

TABLE 3

CLINICAL SYMPTOMS OF INTOXICATION ½, 1, 2 AND 3 HOURS AFTER THE INTAKE OF ALCOHOL. EXPERIMENTS: SEE PREVIOUS TABLES. CONTR. = BEFORE INTAKE

Symptom	Subject N:o 1																
	Contr.	Exp 2				Exp. 4				Exp. 5				Exp. 7			
		½	1	2	3	½	1	2	3	½	1	2	3	½	1	2	3
Smell of alcohol in breath	−	+	+	+	+	+	+	+	+								
Pulse frequency/min.	72	100	104	96	92	78	82	90	92	76	96	88	94	88	76	76	86
Colour of the face	+	+	+	+	+	+	+	+	+	++	++	++	+	++	+	+	±
Walking test, *eyes* open ..	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
» » » closed	0	1	2	0	0	0	2	1	1	1	2	1	0	2	2	0	0
Turning.....	0	0	1	0	0	0	2	1	0	0	0	0	0	1	2	0	0
Romberg, eyes open	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
» » closed	1	1	2	1	1	1	2	2	2	1	1	0	0	1	2	1	0
Finger-finger test	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0
Match test	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
Speech and behaviour	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Subtraction test	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Relative sum of clinical symptoms	1	3	8	2	1	1	7	4	3	2	4	1	0	4	9	2	0

Table 3 cont.

Symptom	Subject N:o 2																
	Contr.	Exp. 2				Exp. 4				Exp. 5				Exp. 7			
		½	1	2	3	½	1	2	3	½	1	2	3	½	1	2	3
Smell of alcohol in breath	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pulse frequency/min	77	92	78	88	68	80	78	76	72	80	82	80	72	100	90	88	80
Colour of the face	+	++	+	±	++	+++	+++	+++	++	++	++	++	++	++	+++	++	++
Walking test, eyes open ..	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
» » » closed..	0	2	2	0	0	0	1	0	0	0	1	1	1	1	1	1	1
Turning.....	0	1	1	1	0	0	0	1	0	0	1	1	1	0	1	0	0
Romberg, eyes open	0	1	2	1	0	0	1	1	0	0	0	0	0	1	2	1	0
» » closed	1	3	3	2	2	1	1	2	2	1	1	2	1	2	2	2	1
Finger-finger test	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
Match test	0	1	2	2	1	0	0	0	0	0	0	0	0	1	2	0	0
Speech and behaviour	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Subtraction test	0	2	2	1	0	0	0	2	1	0	1	0	0	2	1	1	
Relative sum of clinical symptoms	1	12	14	7	3	1	4	6	3	1	5	4	3	8	10	5	2

Table 3 cont.

Symptom	Subject N:o 3																
	Contr.	Exp. 2				Exp. 4				Exp. 5				Exp. 7			
		½	1	2	3	½	1	2	3	½	1	2	3	½	1	2	3
Smell of alcohol in breath	–	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+
Pulse frequency/min.	90	108	102	96	100	108	104	116	116	106	98	100	106	106	92	86	88
Colour of the face	++	+++	+++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Walking test, eyes open ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
» » » closed..	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0
Turning.....	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Romberg, eyes open	0	1	1	1	1	1	0	1	1	0	1	1	0	1	1	1	0
» » closed	1	2	2	2	2	1	1	2	1	1	1	1	1	1	1	1	1
Finger-finger test	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0
Match test	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Speech and behaviour	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
Subtraction test	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Relative sum of clinical symptoms	1	3	4	3	3	2	3	6	3	1	3	2	1	3	5	2	1

jects. This shows that when calcium chloride was taken per os, the alcohol could not be proved to have any effect on the serum calcium values.

4. Slight but evident increases of the same type were observed in the serum calcium in test subjects 2 and 3 in experiments 6 and 7 and in test subject 1 in experiment 7. In experiment 6 test subject 1 showed an obvious increase, when injected a double amount of calcium chloride 20 minutes after the injection, but even this was relatively slight. This proves that calcium chloride, even when injected, is very quickly eliminated from the blood, and the alcohol did not seem to affect the serum calcium values in any way.

II. BLOOD ALCOHOL

The blood alcohol curves of each test subject in all alcohol experiments were very similar type. Calcium chloride, given per os or intravenously, did not seem to have any effect on the blood alcohol values.

III. CLINICAL STAGE OF INTOXICATION

To make the differences between the symptoms clear, the numerical values obtained in different experiments at various times were added together and the relative sums thus obtained were compared (Table 3). The smell of alcohol in breath, the pulse and the congestion of face were not included in the sums, since these symptoms cannot be considered to indicate anything about the degree of the intoxicated.

When alcohol was taken immediately after the dose per os of calcium chloride, the symptoms were found to be slighter than when alcohol was taken without calcium chloride. Calcium chloride, given intravenously, had no effect on the symptoms of the stage of intoxication one hour after the ingestion of alcohol and 20 minutes after the calcium chloride injection, but 2 and 3 hours after the ingestion of alcohol the symptoms of intoxication were milder than when alcohol was taken without giving calcium chloride. The possible reducing effect of calcium chloride on the symptoms of intoxication had in this case been delayed.

According to this study calcium chloride, given in therapeutic doses, cannot be considered to show any peculiar characteristics due to the molecular compound formed by it and ethyl alcohol.

The reducing effect, if any, of calcium chloride on the clinical symptoms of intoxication should be studied by methods allowing less scope for possible errors due to the examiner's subjectivity, to the compensation and influence of practice of test subjects in successive experiments (3). It would also be interesting to find out in what manner other calcium salts, such as gluconate and lactate, affect these symptoms.

SUMMARY

Calcium chloride was given per os and administered intravenously in therapeutic doses to three test subjects, to whom 1 g per kg of body weight of alcohol was given simultaneously. Serum calcium and blood alcohol determinations and clinical examinations of the stage of intoxication were made at various times after the ingestion of alcohol. The following conclusions could be made:

1. No effect of alcohol on the serum calcium values could be established, nor did calcium chloride affect the blood alcohol values.

2. Calcium chloride possibly had a reducing effect on the symptoms of intoxication. This may be due to the effect of calcium chloride on neural functions.

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FROM THE KUMPULA STATE HOSPITAL FOR VENEREAL DISEASES, HELSINKI

RABBIT SYPHILIS AND THE HERXHEIMER REACTION

By

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Penicillin causes a febrile Herxheimer reaction (HR) in early human syphilis. According to Putkonen and Rehtijärvi (1) this reaction begins to appear at the seronegative primary stage, and is regularly present in seropositive primary syphilis. However, the number of cases on which this study was based was small, and, especially as far as seronegative primary syphilis is concerned, the results require corroboration. Sheldon and Heyman (2) examined histologic specimens and found acute inflammatory changes in syphilitic lesions in association with a clinical HR. They observed similar histologic changes in the skin lesions of syphilitic rabbits at the start of treatment, and the same observation was made also by Hume and Turner (3). The work of all these observers stimulated our interest in the HR in rabbit syphilis, which may provide possibilities for studying the HR at the earliest stages of the disease. We confined ourselves to the febrile response and the macroscopic reaction in syphilitic skin lesions.

MATERIAL AND METHODS

Preparation of the Infecting Suspension. — A rabbit was inoculated intratesticularly with the Nichols strain [1] of *Treponema palli-*

[1] The treponema strain was originally supplied by the Venereal Disease Research Laboratory, U.S. Marine Hospital, Staten Island, N.Y. We are indebted for it to Dr. S. Hård, Dermatological Clinic of Karolinska Sjukhuset, Stockholm.

dum and both testes were removed 22 days after inoculation, when eight days had passed from the onset of bilateral orchitis. By the method of Magnuson, Eagle and Fleischman (4), a suspension was prepared from the testes and diluted with normal saline to 30 cc, after which the organisms were enumerated. The number of spirochetes was about 1.5×10^6 per cc.

Test Animals. — Male rabbits, one to two years old and weighing 2 to 2.5 kg, were used for the tests. Twenty-seven rabbits were inoculated intracutaneously in the back at two sites side by side with 0.2 cc of spirochetal suspension containing about 3×10^5 micro-organisms, and intratesticularly (right testis) with 0.5 cc of the same suspension, or about 7.5×10^5 spirochetes.

The Results of the Inoculations. — Observations were made 4, 6, 9, 14, 19, 24, 29, 39, 59, 89, and 179 days after the injections.

STS. — We made quantitative Wassermann (5) and Kahn tests (5, 6) in the Department of Serology and Bacteriology, University of Helsinki, on the day preceding each Herxheimer test and using serial serum dilutions of 1: 1, 1: 2, 1: 4, 1: 8, etc.

Technique of Temperature Measurement. — Because of the instability of the rabbit's body temperature we employed the technique used for determining pyrogenic substances: rabbits were placed in separate boxes (7) and tied up so that their movements were almost completely limited. Before the test, the rabbits were during 14 days habituated to having their temperature taken. During this time they were kept in the boxes first for two hours and later for 4, 6, 8 and 10 hours daily, always in the same room, where the temperature was maintained within the range of 16 to 18° C. The measurements were started at 8 o'clock in the morning, and recorded with half-minute thermometers placed in the rectum 3.5 cm deep for 30 seconds.

The Herxheimer tests were made 5, 10, 20, 30, 40, 60, and 90 days after inoculation. Each test group consisted of 9 rabbits subdivided into 3 groups. The experimental group proper (group 1) comprised 3 syphilitic rabbits and each was given a subcutaneous injection of 2,000 units crystalline G penicillin per kg of body weight in 0.2 cc of pyrogen-free water. Group 2 also consisted of 3 syphilitic rabbits, which were given 0.2 cc pyrogen-free water without penicillin, and the third group of 3 healthy rabbits receiving penicillin like the first group. The syphilitic rabbits generally first

served as controls in group 2 and after this were used for the HR experiment proper. In group 1 there were thus altogether 21 rabbits, and 6 syphilitic rabbits remained in reserve. Food was withheld for two hours before injection and for the whole duration of the test, but the rabbits were allowed a free supply of water. They were placed in the boxes at 8 o'clock in the morning and the temperature was measured 30, 45, and 60 minutes later. The average of these readings was considered the basic temperature of the day. The injections were given at 9 o'clock and temperatures were then recorded at hourly intervals for 24 hours. The syringes were freed from pyrogen by being kept for at least 30 minutes at 250° C and rinsed before the test in pyrogen-free water (8).

RESULTS

Development of Clinical Syphilis. — The cutaneous inoculation was successful in 23 rabbits and failed in 4 rabbits. In the latter, however, the intratesticular inoculation was successful. Thus syphilis developed in all rabbits. The testicular inoculations were successful in 25 rabbits. There was one failure and one uncertain case. This last case was that of a rabbit which died 12 days after inoculation; the testis was then still normal but there was a primary lesion on the back. Table 1 shows when the primary lesions were observed in the rabbits not yet treated with penicillin.

It appears from Table 1 that most of the cutaneous lesions were observed when examining the rabbits after 9 and 14 days and most

TABLE I

DATE OF DISCOVERY OF PRIMARY LESIONS IN RABBITS NOT GIVEN PENICILLIN

Days from Inoculation	Number of Primary Skin Lesions	Number of Rabbits Developing Orchitis
4	0	0
6	0	0
9	13	4
14	13	12
19	6	3
24	2	0
29	0	0
39	2	1
Total	36	20

of the cases of orchitis after 14 days. In reality, however, the incubation periods were slightly shorter because the rabbits were not examined in the interval. Calculating that the signs had appeared midway between these dates of examination, the average incubation period for skin lesions is 12.7 days and for orchitis 12.6 days. It may be added that the rabbits given penicillin before the appearance of primary lesions had average incubation periods of 18.4 days for skin lesions and 26.5 days for orchitis.

On an average the cutaneous lesions increased in size up to the end of the second month from inoculation and then began to heal. When of maximal size, they were about 30 mm in diameter, almost completely covered by a crust. Six months after the inoculation the primary lesions of all 3 untreated surviving rabbits were completely healed.

The syphilitic changes in the testes increased up to the end of the third month. After six months the affected testes of the 3 untreated rabbits just mentioned were free from any signs but so atrophic as to be half the size of the other testis.

All rabbits were dark-field examined and the syphilitic lesions showed a great number of spirochetes. Penicillin administration reduced their number considerably, but 24 hours after it spirochetes were always found if sought sufficiently long.

Serologic Course of Syphilis. — Before inoculation the rabbits usually had negative serologic reactions. Among a total of 46 blood specimens examined, the Wassermann test was only once positive and once doubtful, and the Kahn test positive only once and doubtful in 11 cases. Inoculation with spirochetal suspension made the reaction positive in all cases. Table 2 shows the time lapse from inoculation to seroreversal. The doubtful reactions were classed as negative and the originally positive reactions were classed as turning positive when their titer began to rise.

The serologic incubation period can be calculated from Table 2, by the same method as in Table 1. For the Kahn test it was 14.5 days and for the Wassermann test 24.0 days. The corresponding figures for the rabbits given penicillin were 18.0 and 39.0 days.

On an average, the titers were highest on the 59th day after inoculation: the titer of the Wassermann reaction then ranged from negative to 1: 8 and that of the Kahn reaction from 1: 8 to 1: 64. After this the titers began to fall and after six months all 3 surviving

TABLE 2

DATE OF CHANGE OF SEROLOGIC REACTIONS FROM NEGATIVE TO POSITIVE IN RABBITS NOT GIVEN PENICILLIN

Days from Inoculation	Number of Blood Specimens in which	
	Wassermann Test Turned Positive	Kahn Test Turned Positive
4	0	0
9	0	0
19	6	20
29	8	1
39	1	0
59	2	0
Total	17	21

rabbits had a negative Wassermann test and a positive Kahn test in the dilution 1: 2.

Local HR. — The syphilitic lesions on the backs of the rabbits were examined for any local reactions and measured 6, 12, and 24 hours after penicillin injection. No definite changes appeared which could have been interpreted as Herxheimer reactions.

Febrile HR. — The body temperature of rabbits is extremely sensitive to external conditions. Physiologically it may vary as much as 1.2° C. within 24 hours (9). The average body temperature also differs in different rabbits. For this reason groups of at least three rabbits are used for instance in ordinary pyrogen tests, and a febrile reaction is then defined as a temperature elevation of at least 0.6° C. in two rabbits (8). For the purpose of evaluating the result in our study diagrams were drawn of the average readings obtained in each group of three rabbits at each measurement. These diagrams are shown in Fig. 1.

The figure shows that the curves of the experimental group and of the two control groups are not everywhere on the same level and not quite parallel. Yet the differences are slight and they are not indicative of a febrile Herxheimer reaction. As contrasted with the other groups, the experimental group shows no elevations like those seen in the HR fever curves of human syphilis.

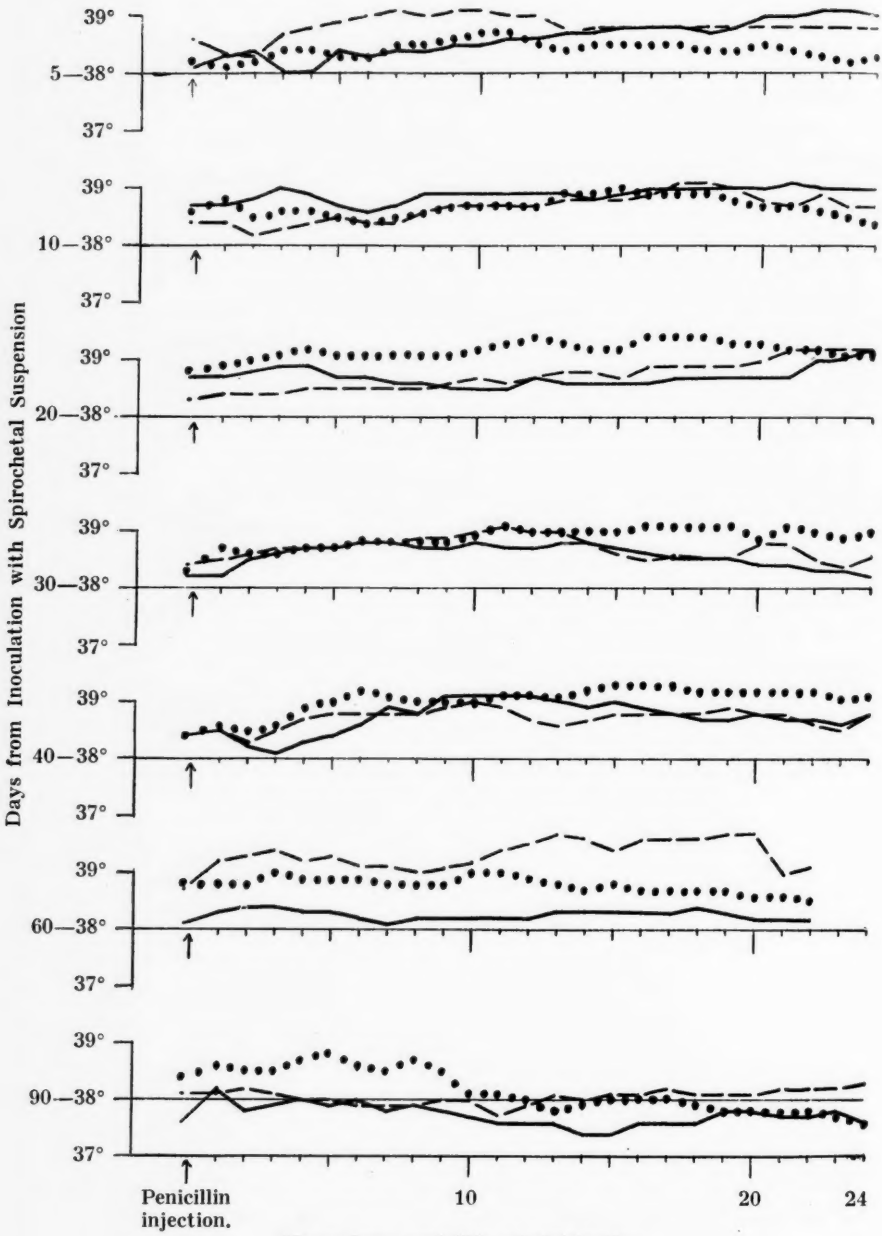

Fig. 1. — Results of temperature recordings in syphilitic rabbits treated with penicillin. Group 1 (experimental group), syphilitic rabbits given penicillin ·····. Control groups: group 2, syphilitic rabbits given pyrogen-free water – – – – –; group 3, healthy rabbits given penicillin ————.

SUMMARY

The writers have studied the occurrence of a febrile Herxheimer reaction and of a macroscopic local Herxheimer reaction in experimental rabbit syphilis. Twenty-seven rabbits were inoculated intracutaneously in the back at two points side by side with 3×10^5 spirochetes and intratesticularly (right testis) with 7.5×10^5 spirochetes. Syphilis developed in all rabbits. The average incubation period was 12.7 days for the cutaneous lesions and 12.6 days for orchitis. The serologic incubation period was 14.5 days for the Kahn test and 24.0 days for the Wassermann test. Each syphilitic rabbit was given a subcutaneous injection of 2,000 units crystalline G penicillin per kg of body weight in pyrogen-free water 5, 10, 20, 30, 40, 60, or 90 days after inoculation. After penicillin administration the rectal temperature of the rabbits was recorded at hourly intervals for 24 hours, using the technique employed in pyrogen determinations. The syphilitic lesions were examined 6, 12, and 24 hours after each injection for any changes indicative of a local HR. Both temperature measurements and inspection of lesions yielded a negative result. This absence of febrile and macroscopic Herxheimer reactions in rabbits is one of the features distinguishing rabbit syphilis from the human disease.

ACKNOWLEDGEMENT

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FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI

SERUM PROTEIN FRACTIONATION IN PYELONEPHRITIS AND NEPHRITIS

By

ANNA-LIISA ALHA

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My studies on the serum protein fractions during pregnancy showed that toxemic pregnant women had very often suffered from pyelonephritis or neprhitis before pregnancy. In this connection it was of great interest to find out whether a renal disease alone could cause changes in the serum protein fractions. In the literature there are comparatively few studies on the serum protein fractions in pyelonephritis and in nephritis, while studies on chronic cases and on nephroses are more numerous.

Rowe observed in 1917 (10) an increase in the serum globulins and a decrease in the albumins in nephritis and in infectious diseases in general. Linder *et al.* (7) (Howe's method) found that in Bright's disease the total proteins usually decreased, whereas the globulins either remained normal or increased slightly. The increase in the globulins was considered to be of a compensatory nature. In a case of nephritis of non-pregnant woman Coetzee (4) (Wu-method) observed a decrease in the albumins, while the globulin content usually remained unchanged except in two cases where there was an obvious increase of the globulins. Thus it was only in a few cases that an increase in the globulins could be considered sufficient to compensate a reduction of the albumins. For reasons mentioned above A/G decreased in most cases. Coetzee observed a slight

increase in the globulins and a slight decrease in the albumins in men suffering from a renal disease. Wiener *et al.* (13) (Kjeldahl-method) noticed the same changes in acute nephritis as in acute infections, i.e. a slight decrease of the albumins in mild cases, a greater one in severe cases. Bing (3) (Henriques and Klausen's method) observed hyperglobulinemia in acute infections, likewise in two cases of chronic nephritis for which he obtained the following values:

	Tot. Prot. %	Alb. %	Glob. %
Nephritis	7.65—7.60	3.42—3.80	4.23—3.80
Normal cases	6.5 —8.0	3.9 —3.3	1.9 —3.0

Epstein (5) obtained very low serum albumin and total protein values in nephritis. Salvesen (11) published a case of nephritis in which the following values were obtained in the course of a period of 3½ months:

Tot. Prot. %	Alb. %	Glob. %	A/G
8.97—10.73	1.67—2.56	7.10—8.32	0.23—0.36

Longsworth *et al.* (8) carried out an electrophoretic comparative study of the normal and nephrotic serum:

	Tot. Prot. %	Alb. %	Glob. %
Norm. serum	6.92	4.43	2.49
Nephrotic serum	5.21	0.53	4.68
» »	4.53	0.88	3.65
» »	3.14	0.74	2.40

In cases of pathological serum the albumin decreased and the globulin increased.

Stacey (12) made studies on acute infections by the methanol precipitation method of Pillemer and Hutchinson. The results showed a decrease in the albumin compared with normal values. In Finland Krusius and Nikkilä (6) carried out a comparative investigation by means of methanol precipitation — sodium sulfate precipitation — and by electrophoretic methods especially in pathological sera. Their investigation includes many severe cases of nephrosis:

TABLE 1

SERUM PROTEIN FRACTIONATION IN ACUTE PYELONEPHRITIS AND NEPHRITIS

Case No.	Age	Diagnosis	Total Protein %	Albumin %	Globulin %	A/G	Nonprotein Nitrogen mg %
1	27	Pyelonephritis ac.	6.93	3.01	3.92	0.77	45
2	42	»	7.90	3.16	4.74	0.67	45
3	36	»	8.20	4.19	4.01	1.05	45
4	22	»	7.56	4.71	2.85	1.65	40
5	28	»	8.31	3.42	4.89	0.67	40
6	21	»	7.84	4.39	3.45	1.24	45
7	17	Nephritis ac.	9.88	3.98	5.90	0.67	40
8	38	Pyelonephritis ac.	7.00	3.24	3.76	0.86	60
9	25	»	7.50	3.69	3.81	0.97	45
10	42	»	7.90	3.16	4.74	0.67	45
		Means	7.90	3.69	4.21	0.92	45
		Stand. dev.	0.83	0.59	0.87	0.32	

Diagnosis	Elecrtophoresis Alb. %	Elecrtophoresis Glob. %	Methanol Precipitation Alb. %	Methanol Precipitation Glob. %
Nephrosis. Cor incomp...	0.95	5.01	1.61	4.35
Nephrosis. Amyloidosis Asthma bronch.	1.88	3.77	1.94	3.71
Nephrosis. Pyelocystitis	2.78	3.10	2.96	2.92
Nephrosis	1.48	3.05	1.34	3.19
Nephropathia chr., Tumor pulm., Cor incomp.	3.31	6.19	1.84	7.66
Nephropathia chr., Aneurysma aortae, Arthritis	2.48	4.04	2.37	4.15
Nephropathia chr.	0.69	3.65	0.61	3.73
Normal values	4.0—4.5	2.8—3.3	3.88—4.08	3.39—3.67

The albumin values were in all cases considerably lower than normal.

My investigation was carried out by the methanol precipitation method of Pillemer and Hutchinson (9), described in an earlier study (1).

TABLE 2

SERUM PROTEIN FRACTIONATION IN CHRONIC PYELONEPHRITIS AND NEPHRITIS

Case No.	Age	Diagnosis	Total Protein %	Albumin %	Globulin %	A/G	Nonprotein Nitrogen mg %
1	29	Nephritis chr.	7.81	3.90	3.91	1.00	40
2	38	Pyelonephritis chr.	6.56	2.99	3.57	0.84	50
3	34	Nephritis chr.	7.60	2.96	4.64	0.64	40
4	23	»	8.06	4.46	3.60	1.24	40
5	28	»	9.13	4.14	4.99	0.83	40
6	24	»	9.56	3.71	5.85	0.64	40
7	21	»	8.98	4.59	4.39	1.05	45
8	24	»	8.06	4.17	3.89	1.07	40
9	42	»	7.87	4.21	3.66	1.14	45
10	16	»	8.50	4.03	4.47	0.90	45
11	20	»	6.81	3.84	2.97	1.29	45
12	55	Pyelonephritis chr.	7.75	3.79	3.96	0.94	50
13	40	Nephritis chr.	6.59	2.06	4.53	0.45	45
14	42	»	7.03	3.79	3.24	1.17	45
15	41	»	8.75	4.32	4.43	0.98	50
16	17	»	5.70	2.50	3.20	0.68	45
17	24	»	8.81	3.45	5.36	0.64	40
18	35	»	6.81	4.14	2.67	1.56	45
19	35	»	9.88	3.04	6.84	0.44	65
		Means	7.91	3.69	4.22	0.92	45
		Stand. dev.	1.13	0.69	1.03	0.30	

The present series of cases, consisting of 29 women, was divided into two groups: acute and chronic. The first group consisted of 9 cases of acute pyelonephritis and 1 case of acute nephritis (Table 1), the second of 16 cases of chronic pyelonephritis and 3 cases of chronic nephritis (Table 2).

Compared with the normal values obtained in my earlier study (2):

	Tot. Prot. %	Alb. %	Glob. %	A/G
Group 1	7.90	3.69	4.21	0.92
» 2	7.91	3.69	4.22	0.92
Norm. women (40 cases)	7.51	4.19	3.32	1.29

the means show a decrease in the albumin and an increase in the globulin values, consequently a decrease in the ratio A/G.

My series contained one case of chronic nephrosis, which I mention for the sake of comparison. The patient was a 72-year old man. The examination was made every fortnight:

Tot. Prot. %	Alb. %	Glob. %	A/G
7.00—7.75	1.10—1.46	5.90—6.29	0.19—0.23

This case showed a very high degree of hypoalbuminemia and hyperglobulinemia.

SUMMARY

Serum protein fractionations were made by the methanol precipitation method on 29 women suffering from acute or chronic pyelonephritis or nephritis. The investigation showed a decrease of the albumin values and an increase of the globulin values and consequently a decrease in A/G. A very high degree of hypoalbuminemia and hyperglobulinemia occurred in one case of nephrosis.

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FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI

ANTISTAPHYLOLYSIN TITRES (ASTA) IN NORMAL AND PATHOLOGICAL SERA

WITH ESPECIAL REFERENCE TO GYNECOLOGICAL INFECTIONS

By

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Kraus and Clairmont demonstrated in 1900 that the staphylococci produced a hemolytic toxin. Investigations by Neisser and Wechsberg (27) showed that hemolysin was secreted mainly by the pathogenic strains of staphylococci. This observation was confirmed by Fraenkel and Bauman (13), while Julianelle (23) observed that saprophytic strains of staphylococci also were able to produce hemolysin, although to a smaller extent than the pathogenic strains.

In 1910 Neisser and Wechsberg demonstrated that the sera of animals infected with pathogenic strains of staphylococci neutralized the hemolysin produced by staphylococci due to apparently specific antibodies, antistaphylolysin (Asta). This observation was not given particular attention until many years later. In 1935 and international antitoxin unit was fixed (15). And since 1935 the antistaphylolysin reaction has been used for examination of staphylococcal antibodies, in the same manner as the antistreptolysin an antipneumolysin reactions, and a number of investigations have been carried out in this field.

THE ANTISTAPHYLOLYSIN TITRE IN NORMAL SERA

It has been found that the antistaphylolysin content of the sera of healthy adult persons generally is below 1 I.U. per cc of serum,

sometimes a little more, between 1 and 2 I.U. (4, 34). Ipsen (20), like Packalén and Bergqvist (29), found somewhat higher values; in the latter investigation, however, the series consisted for the most part of sera of patients suffering from tuberculosis. It is demonstrated in Packalén's investigation of 1946 that the Asta values of such sera are somewhat higher than those of sera of healthy persons. According to Steen and Schøne (35) approximately 95 per cent of the Asta values of a normal series range between 0—2.8 I.U. per cc of serum and values above 3.5 I.U. must be considered in excess of the average. Westergren (36), who earlier regarded values above 1 I.U. as over the average now gives the figure 1.4 as a limit to correspond with the antistreptolysin value 200. The difficulty in excluding from normal series staphylococcal infections with resulting high titre values can be considered the main reason for the above-mentioned diverging figures for a normal Asta titre. In view of the fact that the results of most investigations indicate a value of 2 I.U., in which our own titration results agree, the said figure has been taken as the limit value at our laboratory.

As a rule, newborn infants have Asta values corresponding to those of their mothers. After some time the titre falls, but rises again with increasing age (1, 3, 4, 37).

THE ANTISTAPHYLOLYSIN TITRE IN DIFFERENT INFECTIONS

Deep-lying staphylococcal infections, such as osteomyelitis, show usually increased titre values (2, 7, 8, 9, 11, 12, 14, 19).

Diverging information is given on values in the case of skin affections. According to Connor and McKie (6) and Ramon, Gomez, Richou and Pannequin (32), the titres exceed normal values, but the majority of investigators have been unable to find any increase in titers (7, 26, 30).

The antistaphylolysin titre has been taken as the criterion on effect in active immunization with staphylococcus toxoid. After such a vaccination values are in general higher (1, 8, 9, 10, 26, 31).

STAPHYLOLYSIN PRODUCTION

Lithander's modification of Casman's method (5), as described by Packalén (28), has been used in preparation of the staphylolysin broth. The staphylolysin broth has been infected with a Strain-

Wood 46 staphylococcus culture, cultivated for 12 hours in a broth (*ph* 7) containing 2 per cent rabbit blood. The substrate is poured into a tall glass retort containing a capillary tube, through which slowly flows a gas mixture consisting of 75 per cent air and 25 per cent carbon dioxide. The gas pressure and speed of flow are controlled by adjusting a water drip leading to the bottle with gas mixture. This system is placed for 48 hours in a thermostat at 37° C. After isolation and controls of hemolysis the cloudy broth is centrifuged for 30 minutes, whereafter it is filtered through a Seitz filter. On account of the absorption of the filter the first 250 cc are rejected after which the remainder is tapped into bottles of 100 cc Staphylolysin prepared in this manner will keep for about three months.

TITRATION METHOD

The technique described by Ipsen (21) for antistreptolysin titrations, and used also by Packalén for titration of antistaphylolysin, has been used.

The patient serum is centrifuged and inactivated by keeping it for 30 minutes in a water bath at 56° C. If not immediately used the serum has been stored in the refrigerator. For titration twofold dilutions of serum are made in 0.5 cc of saline, starting from a dilution of 1: 12.5. To each tube 0.5 ml of staphylolysin is added. The staphylolysin dilution used is determined by titration against a standard antistaphylolysin serum.

In distinction to titration of antistreptolysin in which the degree of dilution for the standard serum starts at one unit, the lowest dilution used in this case is 1/25 units. Thus the amount of antistaphylolysin in the different tubes will be 0.04, 0.032, 0.025, 0.02 0.016, 0.0125, 0.01 and 0.08 units.

After mixing serum and staphylolysin the tubes are carefully shaken and kept in a water bath at 37° C for 30 minutes. To each tube 0.5 ml of a two per cent suspension of washed rabbit red cells are added. Thereafter the tubes are again incubated for 30 minutes in a water bath at 37° C, and kept overnight in a refrigerator at + 4° C.

By reading the method given by Packalén has been followed, where the principle is to compare dilutions of the patients serum and the standard serum yielding a hemolysis of 50 per cent.

In the event the degree of hemolysis in a tube is 40 or 60 per cent it is considered to correspond with a 50 per cent hemolysis. If the hemolysis limit lies between two tubes the probable dilution is interpolated between two tubes in accordance to Ipsen's table; this is done also when the hemolysis of one tube is only 0 or 20 per cent and that of the following tube 80 or 100 per cent.

This method of always comparing the 50 per cent. degree of hemolysis of the investigated serum with the corresponding hemolysis in the standard serum has the advantage of simplifying the calculation of values from the tables, at the same time as it is more correct theoretically (16, 29).

For control of the methods used at our laboratory parallel titrations were performed between staphylolysin and standard serum prepared by us and staphylococcus toxin and standard serum from the State Bacteriological Laboratory, Stockholm. In the control investigations the results corresponded fully with one another.

CONTROL GROUP

A little more than 1,000 routine Asta investigations were performed at the laboratory during 1949. In order to establish the average error 50 sera were taken at random and titrated twice, in the course of which the value $\pm$ 13 per cent was obtained as the average error.

Titrations of 206 sera from different age groups, ranging between 0.5 and 80 years, were performed to establish the normal value. In 90 cases with an average age of 3.4 years the average titre was found to be 0.77 units per ml; in seven cases (7.7 per cent) the titre was $\geqq 2$.

In the second group consisting of 82 cases with an average age of 25.7 years, the average titre was found to be 1.77 units per ml; in 27 (33 per cent) of the cases the titre was $\geqq 2$.

In the last group consisting of 34 cases with an average age of 64 years the average titre was 1.1 units per ml; in two cases (5.8 per cent) the titre was $\geqq 2$.

In accordance with the above an average titre of 1.21 units per ml is obtained for all age groups. The frequency increase for the whole material, considering the limit value 2, is 12.6 per cent. See table I. Considering the average titre value 1.77 in the group

25.7 years the limit value 1.4 given by Westergren (36) seems too low. On the other hand, the limit value 3.5 units per ml given by Steen and Schøne (35) seems too high on the basis of this investigation. Consequently, in view of the above-mentioned results, attention should be given to the age of patients in taking readings. A limit value of 1 for children below 5 years of age, 1.8 for persons between 5 and 60 years of age and of 1.2 units per ml for persons over 60 years of age seems justified. In routine clinical work where most of the patient material can be regarded as belonging to the group with 1.77 as average titre value, figure 2 well defends its place as the normal limit value.

TABLE I

CONTROL GROUPS

	Number of Persons Tested	Average Age	Average Titre	Number $\geqq 2$	Per cent $\geqq 2$
Control groups	90	3.4 years	0.77	7	7.7 %
	82	25.7 »	1.77	27	33.8 »
	34	62 »	1.1	2	5.8 »
Total	206	30.5 years	1.21	36	12.6 %

RHEUMATIC PATIENTS

The investigation series also include 104 hospitalized rheumatic patients with clinically verified diagnosis. In 30 cases of rheumatic fever, with an average age of 37 years, the average titre was 1.03 units per ml, six patients (20 per cent) having a titre $\geqq 2$. In 74 cases of chronic polyarthritis with more or less pronounced arthritis, with an average age of 42 years, the average titre was 1.42 units per ml, 20 patients or 27.2 per cent having a titre $\geqq 2$. Thus in neither group did the average titre value exceed the limit value 2. See table II.

TABLE II

RHEUMATIC PATIENTS

Diagnosis	No. of Patients	Average Age	Average Titre	Number $\geqq 2$	Per cent $\geqq 2$
Rheumatic fever	30	37 years	1.03	6	20 %
Chr. polyarthritis	74	42 »	1.42	20	27.2 »

GYNECOLOGICAL INFECTIONS

Titrations were performed on sera of 90 patients with gynecological infections, divided into groups with acute and chronic infections, The writer is much indebted to Professor A. Turunen, Director of the Women's Clinic, Helsinki, who has kindly made the investigation of these patients possible. The first group chiefly consisted of cases with the clinical diagnoses: *Salpingo-oophoritis, pyosalpinx* and *abscessus Douglasi*. Considering the formation of antibodies, only cases with a history of at least three weeks and not exceeding 2 months, were included in this group. The chronic infection group chiefly consisted of cases with the *diagnosis salpingo--oophoritis chronica* and *parametritis*, including *pyosalpinx* and *pyovarium*.

TABLE III

GYNECOLOGICAL INFECTIONS

Diagnosis	No of Patients	Average Age	Average Titre	Number ≥ 2	Per cent ≥ 2
Acute gynecological infections	61	13.5 years	1.22	10	16.3 %
Chronic gynecological infections	29	31.8 »	2.67	14	48 %

Of 61 acute cases with an average age of 32.5 years, 10 sera (16.3 per cent) had a titre of ≥ 2; the average titre being 1.22 units per ml.

In the group of 29 cases of chronic infections with an average age of 31.8 years, 14 sera (48 per cent) had a titre of ≥ 2 units per ml, the average titre being 2.7 units per ml.

Comparing these two groups, which have about the same average age, the occurrence of positive reactions in the chronic infection group (48 per cent) is higher than in the acute one (16 per cent). The average titre value 2.7 in the former is significantly higher than in the latter 1.2, indicating a possible staphylococcal superinfection in the chronic group. In the literature covering this field there are also some evidences pointing in this direction. For instance, Hermann (17) found in pus of 1,005 adnex tumours streptococci and staphylococci in 11.7 per cent of the cases. Schottmüller

and Barfurth (33) found correspondingly (cases of pyosalpinx and pelvic peritonitis) aerobic staphylococci in 2.5 per cent, anaerobic staphylococci in 3.8 per cent, and *Streptococcus putrificus* and anaerobic staphylococci in 12.6 per cent of the cases. Jaegerroos (22) found anaerobic bacteria, chiefly staphylococci and streptococci, in 65 per cent of 24 Douglas abscesses, and Heyneman (18) found staphylococci in 5.7 per cent of 33 Douglas abscesses.

CONCLUSION

Asta values have been counted on a series comprising 400 cases. The method of investigation was controlled by comparing the Asta values obtained using our standard serum and toxin and the values obtained using standard serum and staphylococcus toxin of the State Bacteriological Laboratory, Stockholm. There was full agreement in the results.

The average error was estimated at $\pm$ 13 per cent.

The normal series consisted of sera from 206 patients divided into three age groups. The age of patients should be given attention in estimating the titrevalues. For children below 5 years of age the limit between normal and pathological values should be drawn at 1 unit per ml, for persons between 5 and 60 years of age at 2 units per ml, and for persons over 60 years of age at 1.2 units per ml. If no division into age groups takes place, the figure 2 can be regarded as a limit value, for avoidance of erroneous conclusions.

A series of sera from 104 rheumatic patients failed to show increased Asta values, on the contrary the values must be regarded rather as low.

A series of sera from 90 patients suffering from gynecological infections, divided into chronic and acute infections, showed for the former an average titre value of 2.67 units per ml, and titres of $\geqq 2$ units per ml in 48 per cent of cases, whereas the average titre value for the latter was 1.22 units per ml, with titres of $\geqq 2$ units per ml, in only 16.3 per cent of the cases. The increased titre in 48 per cent of cases indicates possibly a frequent staphylococcal superinfection in the chronic cases.

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THE CYTOLOGY OF THE NUCLEUS SUPRAOPTICUS OF THE RAT

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An abundance of experimental evidence has demonstrated the importance of the supraoptic nucleus in the regulation o water balance in mammals (22). It has been seen that destruction of the supraopticohypophysial system or of a part of it is followed by diabetes insipidus (11). On the other hand, the secretion of the antidiuretic hormone can be experimentally increased by emotional stresses (20), by direct stimulation of the tractus supraopticohypophysius (14), by injecting acetylcholine into the supraoptic nucleus (23), or by intraarterial injections of hypertonic solutions (32).

The magnocellular hypothalamic nuclei are structurally well defined and they can be found throughout the vertebrate series (5). Cytological investigations have indicated peculiar characteristics in the cellular pattern of these nuclei. Multinucleated nerve cells, endocellular capillaries, and acidophilic granules or droplets have been described in the nucleus supraopticus of numerous animals (1, 2, 4, 5, 21, 26—31). Cytological manifestations of so-called neurosecretion have been extensively studied by Scharrer and Scharrer. The reader is referred, for particulars and earlier investigations, to their recent reviews (29, 30) and to the treatise by Roussy and Mosinger (26).

In the study of the rather extensive literature, which will be only partly cited in this paper, it appeared that the supraoptic nucleus of the rat has not been an object of detailed cytological study. Taking into consideration the importance of the rat as

an experimental animal, such a study seemed to be indicated. The purpose of the present investigation is to describe the structure of the nerve cells in the nucleus in question, with reference to the peculiarities described by earlier writers in homologous nuclei of other animals. Special emphasis is laid on the critical evaluation of the staining techniques, a point surprisingly little considered hitherto in connection with the present topic. In addition, some quantitative observations on the supraoptic nucleus are given.

MATERIAL AND METHODS

The series comprised about 50 adult rats, both male and female, white and hooded. The animals were killed by decapitation. The brain was rapidly but cautiously removed from the scull and immersed immediately in the fixing solution. Special care was found necessary in the preparation because even slight manipulations of the brain substance were observed to be followed by distinct cytological changes in the regions of the postmortem injury. In some animals the arteries were perfused first with physiological saline and then with fixing agent.

The following fluids were used for fixation: 10 per cent neutral formol, 10 per cent formol-alcohol, 96 per cent alcohol, 5 per cent trichlorolactic acid, Zenker's, Bouin's, Carnoy's, Maximow's and Regaud's fluids (25). After appropriate fixation, the brains or parts of them were embedded in paraffin through alcohol and benzene. Frontal sections cut at 2 to 20 micra were brought into water and stained with the following techniques: Pischinger's buffered methylene blue at *p*H 4.6—4.7 (25), Einarson's gallocyanin-chrome alum at *p*H 1.2 (7), gallamin blue (6), naphthazarin (6), azan (25), azocarminelight green (24), Masson's trichrome stain, original (25) and modifications (3,33), Hyden's ponceau-duponol-hydrochloric acid method (15), Weigert's haematoxylin (25), haematoxylin-Van Gieson (25).

Nuclear diameters were determined from camera lucida drawings, magnified 2500×. Only nuclei having their centers in the section were measured. The longest (a) and the shortest (b) diameters of elongated nuclei were measured. Thereafter, length-breadth indices (a/b) and corrected diameters ($\sqrt{ab}$) were calculated. The diameters of nucleoli were similarly determined. The number of nerve cell nuclei and nucleoli per cu mm of tissue in the supra-

optic nucleus were estimated by drawing all nuclei and nucleoli including their fragments, and by drawing and measuring planimetrically the area of the supraoptic nucleus visible in the section. The number of nuclei or nucleoli per cu mm of fixed tissue was calculated with aid of the following equation, slightly modified from that of Floderus (12): $N = \frac{a}{a + d - 2h} n$, in which a = thickness of the section in micra, d = mean diameter of nuclei or nucleoli in micra, h = vertical height of the smallest discernible nuclear or nucleolar fragment in micra, n = number of nuclei or nucleoli counted per cu mm of tissue. As measurement of h proved to be difficult, n was counted from sections with different thicknesses (2, 4, 6, 8, 10 micra), and N was calculated with aid of a pair of equations by eliminating the h. About 4000 nuclei with their nucleoli were drawn.

OBSERVATIONS

Quantitative Characteristics of Nerve Cell Nuclei. — The frequency distribution of nuclear diameters in the supraoptic nucleus is presented in table 1. In a curve drawn with the aid of these

TABLE 1

PERCENTAGE DISTRIBUTION OF NUCLEAR DIAMETERS IN THE NUCLEUS SUPRAOPTICUS OF THE RAT

Diameter (micra)	Percentage
6.41— 6.80	0.00
6.81— 7.20	0.57
7.20— 7.60	**3.43**
7.61— 8.00	3.13
8.01— 8.40	5.99
8.41— 8.80	12.29
8.81— 9.20	17.44
9.21— 9.60	**21.73**
9.61—10.00	18.58
10.01—10.40	8.86
10.41—10.80	4.56
10.81—11.20	2.84
11.21—11.60	0.51
11.61—12.00	0.06
12.01—12.40	0.00

values, it was observed that the distribution was not ideal, the curve having two tops. As it appeared possible that these two maximums were due to two different nuclear populations, the sections were examined for cytological differences between nerve cells having large and those having small nuclei. It was found that a part of the cells with small nuclei, called »dark» cells in the following description, differed from the other (»light») cells in having a greater affinity to both acid and basic dyes (see below). By new measurements it was found that the *mean nuclear diameters* of these two kinds of neurons differed highly significantly from each other. The mean with the standard error of the mean for the light cells was 9.3 ± 0.04 micra while the corresponding value for the dark cells was 7.8 ± 0.12 micra. The significance ratio (9) was over 12. A difference between the *mean length-breadth ratios* of the nuclei in the light and the dark cells was, likewise, established. The average ratio in the former group of nerve cells was 1.26 ± 0.01, in the latter 1.52 ± 0.06. The significance ratio was about 4 indicating a statistically significant difference between the two groups. The mean percentages of the numbers of the dark and the light cells were 6 and 94, respectively. The *average number of nerve cell nuclei* per cu mm of tissue was 120,000 as calculated with the aid of the above-given equation. This figure is of the same order of magnitude than those obtained in similar estimations from endocrine glands (12). The total volume of nuclear mass comprised about 5 per cent of the tissue of the nucleus supraopticus. The position of the nucleus in the cytoplasm was as a rule excentric.

Quantitative Studies on the Nucleoli. — Differences consistent with those in the nuclear diameters were shown in the *diameters of the nucleoli* in the two kinds of nerve cells. The mean diameter of the nucleoli in the light cells was 2.8 ± 0.07 micra and that of the dark cells 2.5 ± 0.01 micra. The difference of the means is statistically significant, the significance ratio being over 4. The nucleolar diameters in the nucleus supraopticus were by a qualitative examination easily observed to be distinctly much greater than those of nucleoli in any other nerve cells visible in the frontal sections except for the cells of the paraventricular nucleus. The *ratio of nucleolar volume to that of the nucleus* as calculated from the means of nuclear and nucleolar diameters was in the group of light cells 2.6 per cent and in that of the dark ones 3.3 per cent.

Because individual values of relative amounts of nucleolar mass in each nucleus were lacking, it could not be ascertained whether this difference is statistically significant.

The calculations were made supposing that each nucleus contains only one nucleolus, which, however, is not true. The *average number of nerve cell nucleoli per cu mm of tissue* was 142,000, which is about 18 per cent over the corresponding number of nuclei. Of 2,150 drawn nuclei, on the other hand, 1,371 apparently contained one nucleolus, 121 two, 4 three and 1 four nucleoli. The ratio of the number of polynucleolated nuclei to those containing one or more nucleoli is in this case only 8.4 per cent. The smaller figure is due to the fact that many binucleolated nuclei were represented in the sections by a fragment containing only one nucleolus. The great difference of percentages obtained with the two methods suggests that the position of the nucleoli in the nuclei is excentric, thus favouring the splitting of the nuclei containing two nucleoli in such a way that both fragments contain one nucleolus. It could, indeed, be stated that the majority of nucleoli were situated close to the nuclear membrane. The numbers of nucleoli centrally located and of nucleoli with an excentric position in the nuclei having their centers in the section were counted from the drawings. The designation »excentric position» means that the center of the nucleolus is outside the circle having the center of the nucleus as a center and having a radius which is one half of that of the nucleus. 63 per cent of the nucleoli were thus observed to have a peripheral location in the drawings of nuclei. By calculations, which will not be repeated here, it was found that even if all nucleoli were attached to the nuclear membrane, only about 60 per cent of them should have an excentric position, as defined above, in the drawings, supposing that the distribution of the directions of the peripherally located nucleoli is even. In consequence, the conclusion can be drawn that *practically all nucleoli were attached to the nuclear membrane.*

Intracytoplasmic Capillaries. — The nerve cells formed over one half of the total mass of the supraoptic nucleus. Besides them, a rich network of capillaries and relatively few glia cells were to be seen. Every nerve cell was in the immediate neighbourhood of at least one capillary. The vascular pattern was especially clearly evident in specimens fixed with Maximow's fluid and stained

with azan, azocarmine —light green or with one of the Masson stains, with which the capillaries stained brilliantly. The capillaries, although uncoloured, could be seen quite distinctly with a stereomicroscope even in sections stained, e.g., with methylene blue or gallocyanin. Because observations have been reported according to which there are endocytoplasmic capillaries in some supraoptic neurons of other animals, painstaking efforts were made to find such capillaries. Sometimes, especially in thick sections, it seemed that such a phenomenon was established but this was always found to be only apparent and due to capillaries lying above or below the cytoplasm of the nerve cell. The stereomicroscope magnifying up to 750× was very useful in this kind of study. Taking into consideration the uncommon neurocellular density and the rich vascularization in the nucleus supraopticus it is understandable that pseudoendocellular capillaries were not very rare.

Binucleated Cells. — For the same reason, nerve cells apparently containing two nuclei were occasionally seen. Even these were always found to be illusionary, the sometimes very suggestive image being caused by two superimposed but, as was clearly seen with the stereomicroscope, separate cells. The excentric location of nuclei in the cytoplasm favoured the appearence of pseudobinucleosis.

Stainability of the Nerve Cells. — Of the staining procedures listed in the relevant section the theoretical basis of the following methods is best known: methylene blue (34), gallocyanin (7, 8), ponceau-duponol-hydrochloric acid (15). The results with these stains are, therefore, first reported. Both *the gallocyanin method* and *the methylene blue stain* gave selective and reproducible results. Because progressive staining methods were used without »differentiation» or use of mordants the staining was not influenced by subjective factors. Regarding contrast the gallocyanin stain was the superior of the two methods. Hollborn's gallocyanin, specified by Einarson, was not available but Grübler's similar product served well, while samples from other manufacturers tested gave less good results. The methylene blue method was more convenient than the gallocyanin stain in being rapid. Neither displacement nor weakening of the colour during dehydration in alcohol could be found in the sections fixed in 5 per cent ammonium molybdate for 5 min. after the staining. The contrast, which was weak in

Fig. 1. — Nerve cells in the supraoptic nucleus of the rat. Pischinger's buffered methylene blue stain. Obj. 40×, oc. 15×.

Fig. 2. — High magnification of the same section as in Fig. 1. Obj. 120×, oc. 15×.

unfiltered light, was satisfactory if a yellow filter was used. By varying the acidity of the solutions it was observed that only nuclei and Nissl substance stained with methylene blue if the *p*H was lower than 4.7 and with gallocyanin below *p*H 1.2, as measured with a glass electrode. Only materials which stained under these circumstances are called basophilic in the following description.

Owing to *differences in basophilia*, two extreme types of nerve

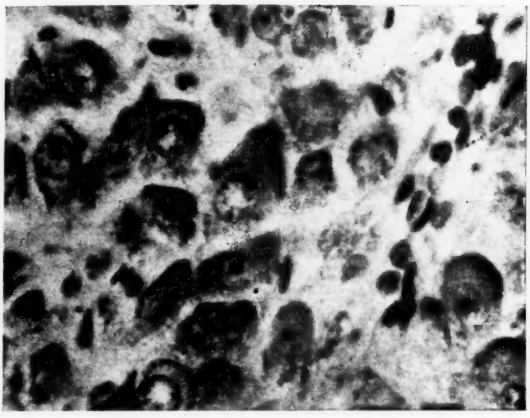

Fig. 3. — Nerve cells in the same supraoptic nucleus as in Figs. 1 and 2. Einarson's gallocyanin — chrome alum stain. Obj. 40×, oc. 15×.

Fig. 4. — High magnification of the same section as in Fig. 3. Obj. 120×, oc. 15×.

cells, designated light and dark cells, were distinguishable. As stated earlier in this paper, *the light cells* had larger nuclei and nucleoli than the dark ones. They were also otherwise larger but stained less intensely with basic dyes than the latter types of cells. The nucleolus was intensely basophilic in both types of cells but the basophilic cytoplasmic material in the light cells was concentrated in the periphery of the cell, as can be seen in Figs. 1 and 2. The central parts stained weakly or they remained un-

coloured. In comparison to other nerve cells visible in a frontal section, the concentration of basophilic material in the periphery of the nerve cells of the supraoptic nucleus was very high. Except for the nucleolus or nucleoli, which had rough borders, and the chromatin threads, the nucleus was pale in the light cells, and even in surface. The cytoplasmic basophilic substance was usually found in the superior border of the cytoplasm, i.e. in the half of the cell nearer the upper surface of the brain. Whether this phenomenon, which is evident in Figs. 1—4, is or is not due to diffusion currents during the fixation, is unclear. Occasionally, small amounts of basophilic materials were observed close to the nuclear membrane but definite nuclear caps were very rare.

The dark cells stained definitely deeper with basic dyes than the light neurons. The cytoplasm and especially the nucleus were diffusely stained. The cytoplasm contained, however, darker granules in the periphery. The form of the nucleus was frequently irregular due to folding of the nuclear membrane. The basophilic material in the nuclei of these cells stained metachromatically purple with methylene blue. The smaller the nuclei were, the more intense was the coloration of the nucleus. The basophilic material in the nucleus was dense close to the nuclear membrane.

Results obtained with gallamin blue, haematoxylin, and naphthazarin were essentially similar to those with methylene blue and gallocyanin.

The image visible in the sections stained with *the ponceau technique* closely resembled that obtainable with basic stains, Figs. 5 and 6. The nucleoli, chromatin material and peripherally arranged granules were stained. To obtain some idea of the spatial relations between substances stainable with ponceau and the basophilic materials, some sections were stained with buffered methylene blue and mounted in an acetate buffer of *p*H 4.6, omitting the fixation of the dye. Some nerve cells in sections thus stained were carefully drawn with the aid of a camera lucida. The stain was then removed by washing in 96% alcohol, brought into water and stained with ponceau. By drawing the same cells again and comparing the new drawings with those drawn earlier it could be observed that the two illustrations of each cell were approximately identical with regard to the distribution of stained materials. In addition to the peripherally located material, of

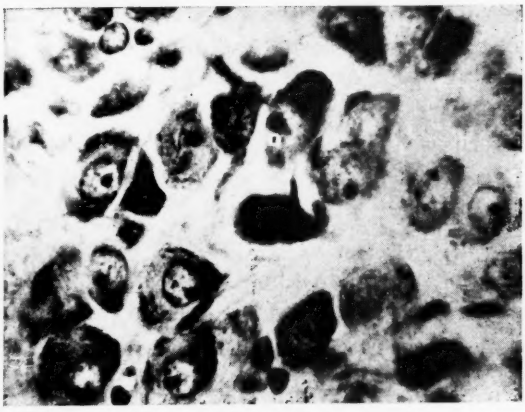

Fig. 5. — Nerve cells in the same supraoptic nucleus as those in the foregoing illustrations. Hyden's ponceau-duponol-hydrochloric acid technique. Obj. 40×, oc. 15×.

Fig. 6. — High magnification of the same section as in Fig. 5. Obj. 120×, oc. 15×.

distribution similar to the basophilic substances and intensely stainable with the ponceau-fuchsin, variable coloration was seen in the more central parts also. The value of this observation is restricted by the fact that, in our hands, the ponceau technique did not give results as reproducible as the methods with basic stains, because of some uncontrolled loss of stain during the treatment of sections after staining. The dark cells stained

with ponceau very strongly (Fig. 5), some cells being entirely red throughout.

Intermediate forms between the light and the dark cells were found.

Before description of the results obtained with *the other staining methods* some general statements on these will be made. In most of them at least one step is poorly defined or subjectively controlled, e.g. by »differentiation until only nuclei are stained». By slightly modifying the length of treatment in different fluids essentially different results may be obtained. In the same section, some red blood cells may appear red while some others are green. By properly adjusting the technique no such divergences appear in the stainability of erythrocytes; unless previously proved, however, there is no certainty that identical substances could not stain differently, *e.g.* due to differences in their concentrations in the section or in their physical state. This is especially true for the methods in which only acid dyes are used, such as the azan stain. The classic staining techniques are of immense value for differential staining of previously morphologically defined structures but special caution is necessary in using different colours obtained with them as indications of the different chemical nature of dissimilarly stained substances ((19, 34).

In sections stained with the azan method, the colour of the nuclei and cytoplasm was dependent on the lengths of time for which the specimens were immersed in different solutions. The same is true for the azocarmin — light green stain. With both these methods, however, the regions containing basophilic materials and those stainable with the ponceau technique stained more or less red. Largely similar results were obtained with the Masson stains. In the first step, iron haematoxylin stained the basophilic materials but in the subsequent treatment at least a part of them coloured red with the ponceau-fuchsin. In the van Gieson method, likewise, regions containing basophilic materials including the nucleoli and coarse Nissl bodies coloured variably red with fuchsin. Thus, with all these methods a part of the basophilic materials, especially dense accumulations of them, stained red, another part staining with the other dyes included in the process of staining. Rather great variations in the intensity of red colour in each individual cell in the same section stained with one of the azocarmine

techniques were noticeable. Some cells were entirely red while others contained only some red material in the peripheral regions. Taking into the consideration that physical adsorption has been said to be the major factor in these kinds of staining processes (34), the interpretation of this observation is difficult.

Outside the nerve cells no definite acidophilic colloid drops or granules were seen. The nuclei of glia cells, erythrocytes and cytoplasmic fragments of nerve cells, staining red with the multicolour stains, simulated, however, such materials.

DISCUSSION

Hyden (15), who studied various nerve cells with ultraviolet absorption microspectrography besides staining methods, found a correlation between the nucleic acid content and the stainability with basic stains on the one hand, and between the concentration of basic proteins and the intensity of colour obtained with acid dyes on the other. Lagerstedt (16—18), similarly using Caspersson's (3) technique, observed in many organs that it is possible to stain selectively nucleic acid-containing protein materials with gallocyanin — chrome alum in acid solutions. Due to the progressive nature of the staining process even quantitative estimation of basophilia was found possible. The correctness of Einarson's (6—8) opinions of the specificity of the gallocyanin — chrome alum stain for the tissue nucleoproteins was thus significantly proved. In the light of these facts it may be probable that the substances called basophilic in this study contain nucleic acids and that some conclusions on the nucleoprotein content in the nerve cells of the supraoptic nucleus are possible with the aid of the methylene blue and gallocyanin methods used.

Two extreme types of nerve cells were observed in this nucleus. In the light cells the cytoplasm and the nucleus apparently contained less nucleic acids than the corresponding structures in the dark cells. The light cells had, further, peripherally located nuclei and peripheral distribution of materials having the stainability characteristics of nucleoproteins. The nucleoli were large and close to the nuclear membrane. Such cells have been found in many conditions, both normal and pathological. For earlier literature the reader is referred to the detailed reviews by Einarson (7) and

Hyden (15). Without listing the conditions here, it is interesting to note that, according to Hyden (15), there is in all such states an intense requirement of cytoplasmic proteins, the synthesis of which is essentially dependent on the function of the nuclear and nucleolar apparatus. In the nucleus supraopticus, the large size and the close connection with the nuclear membrane of nucleoli may be an indication of intensive activity of the nucleolar apparatus, although perinuclear accumulations of basophilic and acidophilic materials were only rarely observed. The dark cells contained these kinds of substances, especially acidophilic materials, in high concentrations. Whether the two types of nerve cells represent different functional stages of similar neurons or are really different in nature and function, remains to be answered. The former possibility seems more attractive to the present writer. In any case, it appears evident on the above-discussed cytological basis that *the nerve cells of the supraoptic nucleus differ from most other neurons in the normal state regarding their protein metabolism.* Many phenomena (see below) can be explained by supposing that the protein breakdown is exceptionally vivid in these cells, a view supported by the presence of rich vascularization in the nucleus supraopticus. In this connection it is of special interest that the cytoplasm of the nerve cells in the supraoptic and paraventricular nuclei of the rat possesses a very intense acid phosphatase activity (10).

As was mentioned in the introduction, many investigators have prescribed secretory functions to the nerve cells in question (1, 2, 21, 26, 27—31). Migration of secretory granules through the supraoptico-hypophysial tract into the neurohypophysis has, further, been supposed on a histological basis (1, 2, 21, 27, 30). If such a migration and continuous transfer of proteins (related to the antidiuretic hormone?) should really occur, the presence of signs of irritation in the responsible nerve cells would be quite natural. In a superficial examination the present writer was, however, unable to establish such migration in rats.

The presence of materials stainable with acid dyes, even in the methods used by Scharrer, were observed in the supraoptic nerve cells of the rat. These substances were approximately similar in distribution in sections stained with the ponceau method recommended by Hyden (15) for the demonstration of basic proteins of histone type, and in specimens treated with the multichrome

stain, used, *e.g.*, by Scharrer. Owing to the importance of merely physical factors in the production of colorations with the latter stains (3), other materials than those having their isoelectric point in the acid side may also stain with them. The close relations of histone-like and nucleic acid containing proteins in the synthesis of cytoplasmic proteins have been demonstrated in nerve cells by Hyden (15). Scharrer *et al.* (31), on the other hand, point out the close relationship between the Nissl substance and the acidophilic neurosecretory material and suppose that the latter is increased with the expense of the diminishment of the Nissl substance. This observation may be partly due to the fact that in multichrome stains there exists a mutual competition between different dyes of the coloration of materials having the same location. Many cytological peculiarities of the nerve cells in the magnocellular hypothalamic nuclei interpreted as signs of secretory function may, however, be also indicative of the presence of large amounts of basic proteins participating in the intense protein synthesis in these cells, (cf. the figures in the papers of Hyden [15] and Scharrer and Scharrer [2, 3]). This assumption, if it will be proved to be correct, by no means invalidates the extensive and carefully performed investigations of Scharrer et al. The fact that acidophilic material in the supraoptic neurons of the rat is interpretable as a necessary part of the protein-synthesizing apparatus does not support, but is neither necessarily in contradiction with, the presence of secretory droplets in the homologous cells of other animals.

The processes designed as neurosecretory are according to Scharrer (28) only faintly visible in the nerve cells of domestic and laboratory animals but very vigorous in those of wild beasts. If the hypothesis presented above holds true, the difference could be explained as follows. The wild animals must, unlike the domestic ones, thirst and drink irregularly, and the regulating center of the secretion of the antidiuretic hormone, the supraoptic nucleus, is severely exercised. In different kinds of experiments, including tests affecting the water metabolism Scharrer and Scharrer (20, 29) failed to show changes in the cytological picture of neurosecretion. The failure may be in part due to the nature of the staining

methods used (see above). Reinvestigations making use of more stable techniques and examining the results from the point of view of the cytoplasmic protein synthesis (7, 15, 18) seem, therefore, to be indicated. Absorption spectrographic investigations, at present entirely lacking for the supraoptic nucleus, would be particularly interesting but even more simple methods such as those used in this study could possibly give some significant hints. The present knowledge of the physiological function of the nucleus supraopticus (see the introduction) suggests that experimental techniques affecting the rate of output of the antidiuretic hormone, e.g. injections of acetylcholine, appear in advance most promising. [1]

In the supraoptic nucleus of the rat, neither true binucleated nerve cells nor intracellular capillaries could be observed. This failure does not, of course, exclude the presence of such peculiarities in homologous cerebral nuclei of other animals.

SUMMARY

The cytological characteristics of the nerve cells in the nucleus supraopticus of the rat have been examined and described. The distribution of basophilic and acidophilic materials, studied with progressive staining methods, as well as the excentric positions of the nuclei and of the exceptionally voluminous nucleoli supposedly indicates an irritation of the protein-forming nucleolar apparatus. Acidophilic materials interpreted by earlier writers as signs of neurosecretory activity are at least partly accounted for by the presence of basic proteins in the vicinity of protein-synthesizing, nucleic-acid containing basophilic structures.

ACKNOWLEDGEMENTS

The writer is grateful to Professor M. J. Mustakallio and to Professor P. Suomalainen for valuable suggestions.

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[1] After this paper was completed, Hillarp's recent article (Acta Endocrinol. 1949, *2*, 33) was seen. He observed definite cytological changes in the supraoptic nucleus of rats injected with hypertonic saline.

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FROM THE DEPARTMENT OF DERMATOLOGY, UNIVERSITY OF PENNSYLVANIA MEDICAL SCHOOL, PHILADELPHIA 4, PA.

STUDIES ON REACTIVATION OF ULTRAVIOLET IRRADIATED VACCINIA VIRUS WITH VISIBLE LIGHT [1]

By

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(Received for publication January 9, 1951)

It has been shown (1, 2, 3) that ultraviolet inactivated bacteriophage remaining in contact with the host cell, can be reactivated under certain circumstances. Some observations seem to indicate that visible light, especially the shorter wavelengths, may act as a reactivator.

This interesting observation encouraged us to try and discover whether it is possible to reactivate other viruses in a similar manner with visible light. We chose a standard egg adapted strain of vaccinia virus for this purpose. The intention was to carry out our programme along the following lines:

1. To find the ideal duration for UV-exposure, which would guarantee a low percentage of surviving infectious units.

2. To treat the UV-inactivated virus *in vitro* with subsequent exposure to various kinds of light before inoculation into eggs.

3. To treat the inactivated virus with visible light *in vivo* through a window in the egg shell, the virus remaining in contact with the host tissue during exposure.

The inactivating effect of ultra violet light on vaccinia virus was determined in a series of preparatory experiments. The Germicidal Lamp (General Electric Inc.) was employed.

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As it was considered important that different parts of the virus suspension should, if possible, be equally exposed to the light, flat Petri dishes (5 cm diam) were used and the surface of the solution was agitated, always in the same manner, a continuously slightly rocking movement being maintained by means of a rotating wheel with a slightly eccentric axis. The dishes were placed in a row under the Germicidal lamp, each of them at the same distance of 15 cm from the light tube. The same strength of virus suspension was used for each exposure, i.e. the stock solution of virus diluted in physiologic sodium chloride solution 1: 10. After the exposures various dilutions ranging from 10^{-2} to 10^{-5} were made in test tubes. To 0.8 ml of the saline solution for each tube, 0.1 ml of a stock solution of penicillin and streptomycin was added so as to make a final concentration of 100 units of penicillin and 10 mgr of streptomycin pro ml.

For quantitative determination of the survival of virus the exposed and unexposed suspensions were inoculated on the chorioallantoic membrane of 8—10 days embryonated eggs, using the false air sac technique. Each egg was inoculated through the hole over the false air sac with 0.05 ml of the specimen, the hole in the shell being subsequently protected from dust by a piece of Scotch tape, in the usual way, folded over the hole so as not to close it too tightly. The eggs were then kept on their sides in the incubator for 48 hours at 35—36°C before being checked.

Using the Germicidal lamp, and keeping it at a distance of 15 cm, the optimal time of exposure for our purposes was found to be 15 sec (see Table I). This time of ultraviolet irradiation was then used in all subsequent experiments. With this amount of UV-light the percentage of surviving infectious units was kept at a suitably low level, amounting to about 0.2 per cent.

The next experiment was to study the effect of diffuse daylight on ultraviolet inactivated virus *in vitro* (Table II).

Half of the ultraviolet irradiated eggs were kept in the dark and the other half of the eggs were exposed for 3 hours to diffuse daylight. Inoculations were then made from dilutions 10^{-2} and 10^{-3} of the ultraviolet-irradiated specimens and from 10^{-4} and 10^{-5} of the controls. No reactivation of virus by daylight was demonstrated. A similar experiment was made using the following sources of artificial light:

1) 200 Watt bulb filament at a distance of 45 cm for 1 hour.
2) The same for 4 hours.
3) Fluorescent tube at a distance of 60 cm for 4 hours.

A control group of the specimens was kept in the dark for 4 hours and another control group was inoculated immediately.

TABLE I

EFFECT OF ULTRAVIOLET IRRADIATION ON AN AGITATED CLEAR SUSPENSION OF VACCINIA VIRUS.

Duration of Ultraviolet Irradiation	Number of Surviving Infectious Units per ml	% of Surviving Infectious Units
0	1.8×10^7	100 %
5 sec.	$>2\times10^5$	—
10 sec.	$>2\times10^5$	—
11 sec.	2.2×10^5	1.2 %
15 sec.	4.2×10^4	0.2 %
19 sec.	2.0×10^3	0.01 %
60 sec.	0	0
120 sec.	0	0

TABLE II

EFFECT OF DIFFUSE DAYLIGHT ON ULTRAVIOLET INACTIVATED VACCINIA VIRUS

Type of Irradiation		Surviving Infectious Units per ml
Ultraviolet	Daylight	
0	0	8×10^5
0	3 hrs.	$<2\times10^5$
15 sec.	0 (darkness)	1.5×10^4
15 sec.	3 hrs.	3.0×10^3

Again UV-irradiated specimens were inoculated in dilutions 10^{-2} and 10^{-3} and the non-irradiated controls in dilutions 10^{-4} and 10^{-5}. Four eggs were inoculated in each group, i.e. 72 eggs in all.

As can be seen in Table III the number of infectious units in the controls was 3.5×10^7 if inoculated immediately. It decreased however to 0.8×10^7 if stored for 4 hours in darkness. Almost the same figures (0.8×10^7 and 1.2×10^7) were observed for controls which without UV-exposure were kept for 4 hours in artificial light from a 200 Watt bulb or a fluorescent tube. The same facts

TABLE III

EFFECT OF ARTIFICIAL LIGHT ON ULTRAVIOLET INACTIVATED VACCINIA VIRUS

Type of irradiation				Number of Surviving Infectious Units per ml
Ultraviolet	200 Watt Bulb Filament	Fluorescent Tube	Stored in the Dark Only	
0	0	0	0	3.5×10^7
0	0	0	4 hrs	0.8×10^7
0	1 hr	0	0	2.0×10^7
0	4 hrs	0	0	0.8×10^7
0	0	4 hrs	0	1.2×10^7
15 sec.	0	0	4 hrs	0.9×10^5
15 sec.	1 hr	0	0	9.8×10^5
15 sec.	4 hrs	0	0	1.0×10^5
15 sec.	0	4 hrs	0	0.6×10^5

TABLE IV

EFFECT OF VISIBLE LIGHT ON ULTRAVIOLET INACTIVATED VACCINIA VIRUS INOCULATED ONTO CHORIOALLANTOIC MEMBRANE

Type of irradiation			Number of Plaques per Membrane (av. of 3 eggs)			
			Dilutions of Inoculum			
Ultraviolet of Inoculum	Incubated in the Dark	Incubated in Light (200 Watt Filament)	10^{-2}	10^{-3}	10^{-4}	10^{-5}
0	48 hrs	0	—	—	ca 100	7
0	0	48 hrs	—	—	ca 100	9
15 sec.	48 hrs	0	40	0.3	0	—
15 sec.	0	48 hrs	20	2	0	—

can be seen in the ultraviolet-irradiated series, in which the controls, stored for four hours in the dark, showed an average of 0.9×10^5 surviving units, while specimens exposed to light from the 200 Watt bulb and the fluorescent tube showed an average of 1.0×10^5 and 0.6×10^5 surviving units, respectively. If the specimen was exposed to the light for 1 hour only and then inoculated without further storage a 10 fold count (average 9.8×10^5 surviving units) was obtained. No reactivation of virus by visible light could be proved.

Working with these experiments a source of error was observed that might be mentioned here: The time during which the diluted virus suspensions remained in the Petri dishes seemed to have an

unfavourable influence, reducing the number of surviving infectious units. In this connection we were unable to ascertain whether this was due to the dilution, the temperature, or to some disturbing factor in the glassware. We tried to eliminate this fault by keeping the controls in the dark and at the same temperature for as long a time as the other dishes were exposed to visible light.

Our next step was to study whether the virus could be reactivated by visible light *in vivo*, when remaining in contact with the host tissue. For this purpose the ultraviolet irradiated specimens and the non-irradiated controls were inoculated on to chorioallantoic membranes and afterwards exposed to visible light through a cellophane (Scotch tape) window in the egg shell. Half of the eggs were incubated in light, the others in the dark. Table IV shows that these experiments, too, gave a negative result.

Starting from the theory that shorter wavelengths may possess a better reactivating power, we decided in addition to try the »Black light» (General Electric Inc.). This light was said to have its greatest energy between 3500 and 3800 Å, but it also has a good deal of shorter violet waves up to 4000 or 4100 Å. It was supposed to have no germicidal or erythemal effect at all. Twelve eggs inoculated with ultraviolet-inactivated virus in dilutions 10^{-2} and 10^{-3} were subsequently exposed to »Black light» at a distance of 25 cm for 30 minutes through a Scotch tape window over the false air sac, half of the eggs only once, the others three times within 8 hours.

When the eggs were checked after 48 hours incubation, it was observed that while there were 36 plaques on an average in the ultraviolet inactivated 10^{-2} controls kept in dark, the »Black light»-treated eggs revealed no plaques whatsoever. About half of the embryos had died, too. Whether this mishap was due to the presence of small quantities of shorter ultraviolet rays in the »Black light», sufficient to injure the virus, is difficult to state on the basis of a single experiment with only a few eggs.

SUMMARY

An egg-adapted strain of vaccinia virus in a clear suspension was exposed to ultraviolet irradiation (Germicidal Lamp at 15 cm)

and studied on embryonated eggs using the false air sac technique. After 15 sec. of irradiation the average percentage of surviving infectious units was estimated at 0.2. An effort was made to reactivate the inactivated virus by subsequent exposure to various lights in vitro as well as in vivo, through a window in the egg shell. Diffuse daylight, 200 Watt bulb filament, fluorescent light and »Black light» were used. No reactivation could be noticed.

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SPONTANEOUS TUMOR CONTAINING ADULT NERVOUS TISSUE IN THE HYPOTHALAMIC REGION OF A RABBIT

By

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(Received for publication September 14, 1950)

Primary nerve cell tumors are relatively rare. In man, about 200 cases have been reported. In most instances they have been extracranial and seem nearly always to have been derived from sympathetic ganglion cells. Willis (18), in his authoritative book on the subject, doubts, furthermore, the presence of real nerve cell tumors in the central nervous system. For further details, see his work.

The common laboratory animals are frequent objects of routine pathological examination. Only a few descriptions of intracranial nervous tumors in them are, however, to be found in the literature. Margulies (11) found a teratoma in a rabbit autopsied at random without preceding symptoms. The tumor was in the infundibular region and contained mucus-filled cysts with cylindrical, partly ciliated epithelium, glands, plain and striated muscle fibers, hyaline cartilage, and connective tissue. Jaffé (7) refers to Shima's case, likewise a rabbit teratoma near the tuber cinereum, showing on histological examination numerous cysts, partly covered with cylindrical ciliated epithelium, cartilage, and various kinds of glands. In addition, there were islets of nervous tissue. In the cerebello-pontine angle of a guinea-pig brain, Lutz (10) revealed a ganglion cell — containing teratoma with muscular, cartilaginous, and glandular tissues.

The present writer came a cross a spontaneous tumor in the hypothalamic region of a rabbit brain. Closer study showed that

it was mainly composed of adult nervous tissue. On account of the rarity of pathological findings like this a description of this case will be justified.

MATERIAL AND METHODS

The rabbit was 6 months old, and weighed 1400 g. The animal had been used as a blood donor. It died during the second heart puncture. About one month previously, 20 ml of blood had been taken. In comparison to other animals of the same black strain and age, this one had grown much more slowly. Otherwise, the rabbit behaved like the others showing, for instance, no apparent motor disturbances.

The brain was removed and fixed for four days in 25 per cent formol neutralized with calcium carbonate, washed with running water, dehydrated in alcohol, and embedded in a wax-paraffin mixture through benzene. The block was serially sectioned at 10 μ. The following staining methods were used: Pischinger's buffered methylene blue (14), Einarson's (2) gallocyanin — chrome alum, Bodian's (1) copper-protargol impregnation, Weigert's iron hematoxylin-vanGieson (14), Regaud's hematoxylin (14), Foot's (5) modification of Masson's trichrome stain, and a combined Masson-Heidenhain stain.

Nuclear measurements were carried out with the aid of camera lucida drawings. Only nuclei which had their centres in the section were drawn. The longest (a) and the shortest (b) diameter of each nucleus were measured, and the length-breadth index ($=\frac{a}{b}$) and nuclear volume ($=\frac{4}{3}\pi\sqrt{a^3b^3}$) calculated.

DESCRIPTION OF THE TUMOR

The location of the tumor below the brain is seen in Fig. 1. The dimensions of the tumor were: length 12 mm, breadth 8.5 mm, and height 11 mm. Macroscopically, the tumor was bean-shaped and its surface was smooth and of the same color as that of the brain. Rostrally, the tumor reached the olfactory bulbs, bilaterally the temporal lobes and posteriorily the pons. It was broadly connected with the basal surface of the brain causing a marked deviation of the hypothalamus and the infundibular stalk to the right. In general, the tumor was sharply demarcated from the normal brain tissue, but a distinct boundary could not always be observed

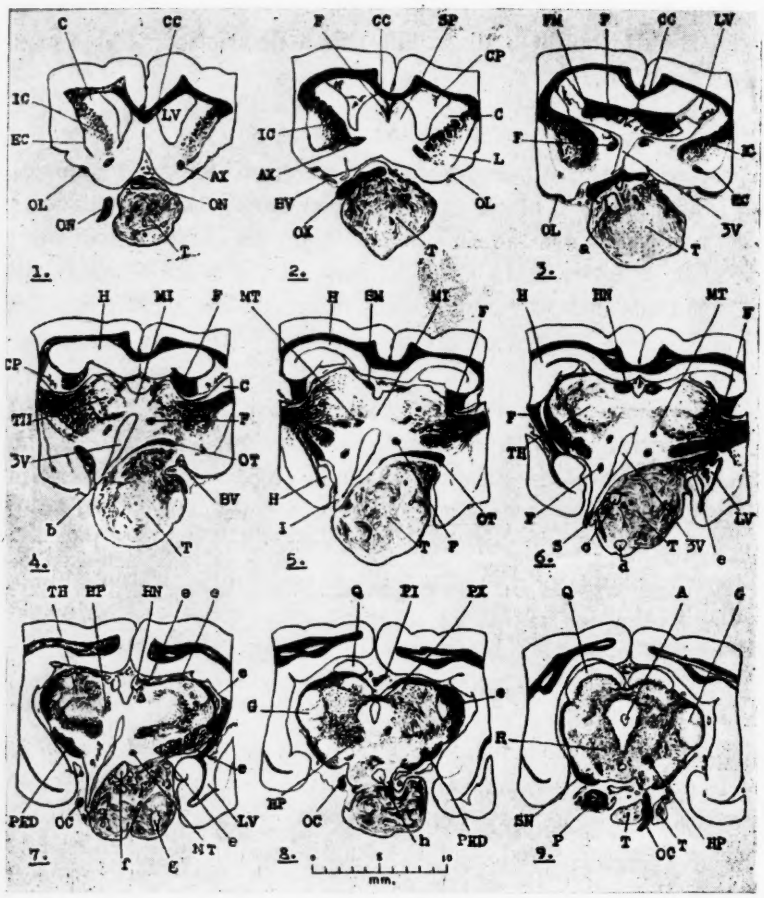

Fig. 1. — Camera lucida drawings illustrating the location of the tumor. The figures represent transverse sections through successive rostrocaudal levels, about 200 μ between each section. A, cerebral aqueduct; AX, anterior commissure; BV, blood vessel; C, caudate nucleus; CC, corpus callosum; CP, chorioid plexus; EC, external capsule; F, fornix; FM, foramen Monroi; G, geniculate body; H, hippocampus; HN, habenular nucleus; HP, habenulopeduncular tract; I, infundibulum; IC, internal capsule; LV, lateral ventricle; MI, massa intermedia; MT, mamillothalamic tract; OC, oculomotor nerve; OL, olfactory tract; ON, optic nerve; OT, optic tract; OX, optic chiasma; P, pons; PED, cerebral peduncle; PI, pineal body; PX, posterior commissure; Q, quadrigeminal body; R, red nucleus; SM, stria medullaris; SN, substantia nigra; SP, septum pellucidum; T, tumor tissue; TH, thalamus; 3V, third ventricle; a, c, d, f, g, h, cysts; b, indistinct demarcation between normal and tumor tissues; e, tumor process reaching the pineal body.

between the hypothalamus and the tumor (*e.g.* Fig. 1, 4 b). For the detailed topography of the tumor in relation to the brain, which will not be described in the text, the reader is referred to Fig. 1. One fact, however, needs special mention. From the left superior corner of the massive tumor a long slender process grew out. In the beginning, it was bordered superiorily by the left optic tract and laterally by the right wall of the left lateral ventricle (Fig. 1, *6* e). The process then ascended round the left side of the mesencephalon up to the pineal body (Fig. 1, *7*, *8* e).

Microscopical Data. — The tumor contained adult nervous tissue as well as hypophysial tissues. It consisted mostly of nervous tissue. Nerve cells and both myelinated and non-myelinated nerve fibers were embedded in a glious stroma (Fig. 2). Nerve cells were found throughout the tumor including the parts containing hypophysial structures. Accumulations of nerve cells resembling cerebral nuclei were observed but otherwise, especially in those parts of the tumor with hypophysial tissues, the distribution of the nerve cells was merely scattered. Nerve fibers, chiefly non-myelinated, were also visible everywhere in the tumor region. As well diffusely distributed fibers crossing each other as some formation of fiber tracts was to be seen. The afore-mentioned tumor process (Fig. 1, *6* e), in its beginning, showed a dense accumulation of interlacing fibers somewhat resembling the arrangement of an amputation neuroma.

The nerve cells in the tumor and their nuclei showed a great variability in size. While the smallest cells had dimensions such as 15×16 and 13×17 μ, the largest ones were found to measure 32×40 or 28×45 μ. Comparative measurements of normal ganglion cells in various parts of the brain showed that their sizes were within this range. Normal nerve cells within one nucleus had, however, a smaller variability. Volumes and length-breadth indices of nuclei of tumor nerve cells were compared with those of the *nucleus ruber*, whose cells were on an average of the same size as the tumor nerve cells. The mean nuclear volumes were of the same order of magnitude in both groups. In 11.5 per cent of the tumor nerve cells the nuclear volume, however, exceeded the volume of the largest measured nucleus in the *nucleus ruber*. This fact was not due to possibly erroneous volumes of nuclei with high length-breadth indices (over 1.15), as was shown in calculations excluding

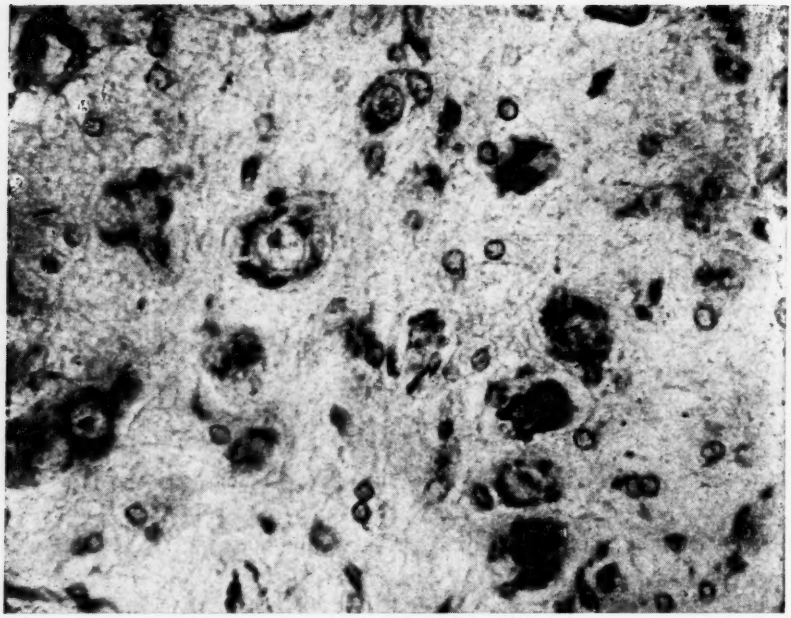

Fig. 2. — Photomicrograph of an accumulation of nerve cells in the tumor. Note variations in the size and appearence of nerve cells. Einarson's gallocyanin-chrome alum. Magnification about 500×.

these nuclei. The length-breadth index was significantly greater in the nuclei of the tumor nerve cells, indicating a more elongated form, than those of cells in the red nucleus (means 1.18 and 1.07, respectively).

The nerve cells in the tumor varied very much in the shape, and in the number of their processes. Uni-, bi- and multipolar cells were found. Most of them were round or multangular but fusiform cells could be seen, also (Fig. 3). The axons were often unusually broad.

The round or oval nucleus was mostly excentrically placed in the cell, as was the nucleolus in the nucleus (Fig. 3). Binucleated cells (Fig. 3, *14*) were rare. In specimens stained with gallocyanin or methylene blue the nucleolus seemed relatively large and of surface uneven. In modified Masson preparations this roughness was found to be due to granular material staining with hematoxylin and surrounding the red fuchsinophil nucleolus. Many nerve

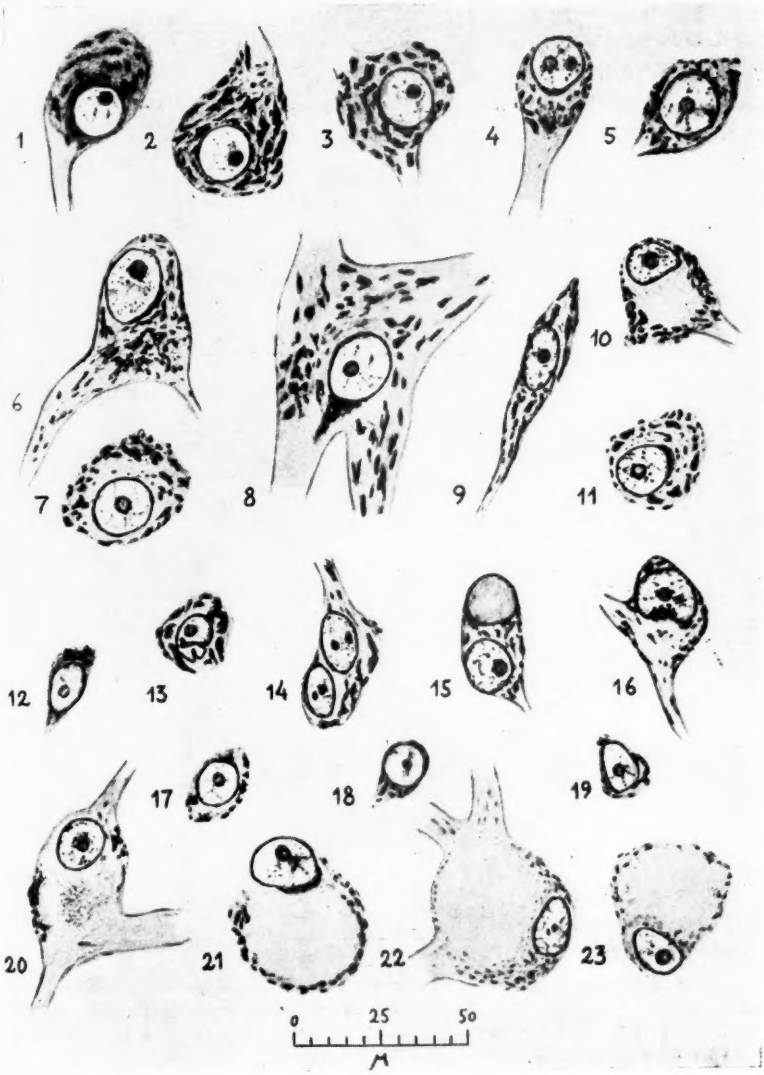

Fig. 3. — Camera lucida drawings of tumor nerve cells stained with Einarson's gallocyanin method. Great variations in size, shape and stainability of the cells are striking.

cells in the tumor showed, not only enlargement in the size of the nucleolus and accumulation of basophil material round it, but a relatively rich content of minute granules and threads staining with gallocyanin, methylene blue or hematoxylin. This chromatin material had to a large extent accumulated adjacent to the nucleolus. Some cells showed folding of the nuclear membrane (Fig. 3, *13*, *16*).

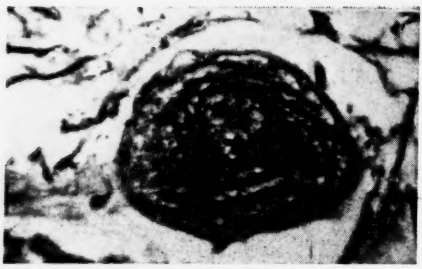

Fig. 4. — Photomicrograph of a tumor nerve cell impregnated with Bodian's technic to show the neurofibrils. These form a dense network in the cytoplasm. Magnification about 600×.

Fig. 5. — Nerve cells from the same section and in close neighborhood with that presented in the foregoing figure. No definite neurofibrils can be seen. In the left, one half of a vacuole having probably remained after liquefaction of a nerve cell.

In the cytoplasm both neurofibrils and Nissl bodies were demonstrable. The neurofibrile made visible with the multiple Bodian technic were, in some cells, densely packed (Fig. 4), while other cells in the immediate neighborhood were found to contain only a few such fibrils or none (Fig. 5). Some examples of gallocyanin stained cells are given in Fig. 3. To use Nissl's (12) morpho-

logical classification, based on the distribution of the chromophil material, gryochrome cells were most common (Fig. 3, *7*, *10*, *11*, *16*, *21*, *22*, *23*). Stichochrome cells were next frequent (Fig. 3, *1*, *2*, *3*); archyo- and karyochrome cells were somewhat rare. All intermediate types were observed. The form of the Nissl bodies and their distribution in the cytoplasm somewhat differed from those seen in most normal nerve cells, being grotesque and irregular. Regarding the intensity of staining with basic dyes all intermediate cell types from »chromoneutral» to »chromophobe» types

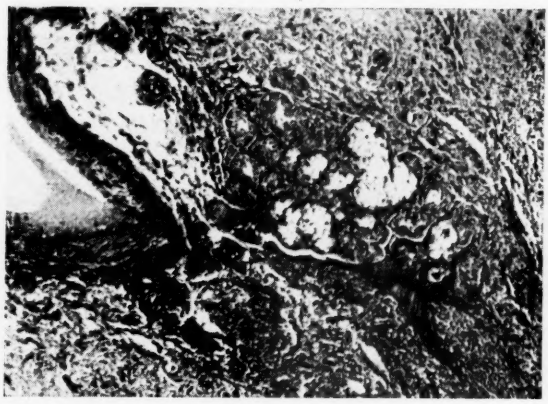

Fig. 6. — A part of one of the numerous cysts is to be seen in the left. In the right side of it a seromucous gland is observable. The mucous cells, appearing uncolored in the photomicrograph, stained metachromatically with methylene blue. Foot's method. Magnification about 20×.

(Einarson (3)) were established but no one »chromophil» cell could be found. Cells with relatively scanty Nissl substance located in the periphery of the cytoplasm were common, *i.e.* chromophobe gryochrome cells. In this particular type of cell, hypertrophy of the nucleolus and accumulation of chromatin material in the nucleus were often obvious. The occurrence of a basophil cap staining with the gallocyanin method was established close by the nuclear membrane in about 10 per cent of such cells (Fig. 3, *4*, *11*, *16*, *21*). No neuronophagia was to be observed. In some cells, acidophilic droplets staining with ponceau-fuchsin with the Foot-Masson technic were seen in the cytoplasm (Fig. 3, *15*); such cells were, however, very rare. The majority of chromophobe cells tended to swell, and there were numerous holes of about 50 to

100 μ in the tumor, probably representing the former sites of liquefied nerve cells.

As mentioned, hypophysial tissues were seen besides the nervous tissue in the tumor region. No distinct demarcation between these tissues could be seen. Cells diagnosed as pituicytes as well as basophil and chromophobe intermediary lobe cells were distinguished. Numerous hypophysial cysts of varying size and nature were characteristic. There were cysts measuring up to 2 mm in diameter (Fig. 1, *3* a). The cysts were covered with either stratified, cuboidal or cilitated cylindrical epithelium and contained colloidal matter. In this, round, vacuolated, partly polynucleated cells were sometimes to be seen. The colloid stained green or red in the Masson preparations and, in some cysts, metachromatically purple with methylene blue, the staining properties varying in different kinds of cysts. In the neighborhood of a cyst a typical seromucous gland was revealed (Fig. 6). The mucous cells of the gland were filled with material staining metachromatically with methylene blue as did the colloid in some, apparently retention, cysts.

The overlying brain was slightly edematous but showed othervise no definite histopathological changes. The Sylvian aqueduct contained blood, however.

Organs other than the brain were, unfortunately, discarded before the hypothalamic tumor was observed.

DISCUSSION

Willis (18) points out that special caution is necessary in diagnosing cells in tumors of the central nervous system as nerve cells because atypical glia cells may closely simulate them. The cells called nerve cells in the foregoing description had the following characteristics of neurons: 1) large vesicular nucleus, 2) cytoplasmic bodies stainable with the gallocyanin — chrome alum stain, which has been said to stain only nucleic acids in histological sections (3, 4, 7), 3) neurofibrils demonstrable in the pericaryons and in the cell processes with Bodian's method, 4) more or less typical form of the cells and their processes. There is, thus, no doubt about the existence of real ganglion cells in the tumor described.

The tumor showed an indistinct boundary with the neighboring

hypothalamic tissues. This fact need not be taken as an indication of malignant growth, especially as the differentiation grade was high in spite of some atypical cells. It may be questioned, indeed, whether the tumor is of real neoplastic nature or merely a hamartoma or malformation. Differential diagnosis is not easy on a histological basis only but the retarded growth of the animal, possibly caused by the disturbing action of the tumor on the hypophysial function, may be an indication of a slow growth of the tumor, which perhaps is some intermediate form between hamartoma and »real» tumor.

With regard to the origin of the tumor, it is of interest that its nerve cells were morphologically much like those described in the vegetative nervous system, hypothalamus and neurohypophysis (15, 16, 17). It may be further notized that acidophil granules, which were seen in some nerve cells in the tumor, have been described in hypothalamic neurons (16) and in autonomic ganglion cells (9).

It must be mentioned, however, that peripheral arrangement of the Nissl substance, nuclear and nucleolar enlargement, folding of the nuclear membrane, accumulation of basophil material in and close to the nucleus, as well as excentric position of the nucleus and nucleolus are indications of an increased activity of the neuron (3, 4, 6). In this particular case, such a cytological picture may have been caused by the tendency of the tumor to grow despite pressure by the surrounding brain tissues which led to a formation of structures resembling an amputation neuroma.

The hypophysial tissues, including glands and cysts, were like those described in Romeis's (13) monograph, the cysts being, however, abnormal large, possibly due to obstruction and retention.

SUMMARY

A spontaneous tumor in the hypothalamic region of a rabbit randomly autopsied was described. The tumor consisted mainly of adult nervous tissue. Typical nerve cells showed neurofibrils and a peripheral arrangement of scanty Nissl substance, and they varied much in shape, size and number of processes. Nerve fibers were mostly non-medullated. Hypophysial tissues, glands and large cysts were seen. Demarcation from the hypothalamus was not always distinct.

ACKNOWLEDGEMENTS

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SACCHARIN AS AN ALLERGIC AGENT

REPORT OF A CASE OF SIMULTANEOUS CROSS SENSITIZATION AGAINST VARIOUS SULFONAMIDE DRUGS AND SACCHARIN

By

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(Received for publication December 27, 1950)

It is well known that SA drugs may produce allergic reactions of many kinds. The literature on this subject, already of notable proportions [1], also contains reports of cases of *cross sensitization*. In most cases this phenomenon has been observed between different SA drugs, but there have also been cases where the other factor in the reaction is a substance not belonging to this group of drugs, and particularly some local anesthetic.

Saccharin [2], on the other hand, has in recent years been regarded as a substance which is physiologically fully harmless, and there is no intimation whatsoever in the textbooks on toxicology and pharmacology of any untoward side-effects from its use.

In fact, the most recent and probably the most complete survey of the investigations on the biological properties of saccharin is to be found in the non-medical literature, i.e. the monograph by Suter (9) on the chemistry of organic sulfur compounds.

As to our knowledge no cases of saccharin allergy have so far been reported, it may be of interest to record the following case, in which the intestinal symptoms produced by saccharin should probably be regarded as an allergic reaction.

[1] A detailed review of the subject is not considered necessary in the present report, especially as a number of fairly recent surveys are available, among which may be mentioned those by Sulzberger et. al. (8) and Croxon Deller (1).

[2] The saccharin preparations used for sweetening contain either free saccharin (o-benzoic-sulfamide) or its sodium salt.

Case Report

The patient, I. L., a 35 year old dentist, had no familial history of asthma, medicinal rash or hypersensitiveness for sweetening substances. A paternal uncle had milk crust as a child and the uncle's son prurigo diathésique Besnier; a paternal aunt suffered from rheumatoid arthritis.

At the age of 1—3 years the patient had milk crust and in 1943 parotitis. In 1946 vitiligo appeared on his hands and gradually spread to the feet, buttocks, around the anus and to the vicinity of the mouth, and later, in association with SA hypersensitivity, to the glans penis. In the past two years the vitiligo around the mouth had diminished to one-half of its former area. The eating of strawberries was sometimes followed by vomiting. There had been no allergy to the novocaine and procaine used in connection with tooth extractions, or to other drugs than those mentioned. — The drinking of alcoholic liquors was always followed by nausea on the following day.

Since 1942 he had, for different diseases, taken a number of times (always per os) various SA drugs, among which he could mention prontosil, sulfanilamide and sulfapyridine. The dosage was generally a daily tablet of 2.5—3.0 g, taken for 2 days.

Prior to 1943 there had been no symptoms of hypersensitivity to SA drugs. In August 1943 complications appeared in connection with treatment (Prontosil rubrum »Merck», 3 g for 2 days). In about 5 minutes the first tablet (0.5 g) caused intense itching, first in the scrotum and the inner surface of the thighs and later also over the right cheekbone, at the left corner of the mouth, at the base of the left thumb and in the glans penis. About a day later reddish patches appeared in these areas, two or three days later they became exudative, and finally a purulent crust was formed. Nausea set in simultaneously with the itching and lasted throughout the period of SA administration. Similar skin symptoms and nausea occurred later in connection with any SA treatment, i.e. in October 1943 from sulfapyridine (6 g in 2 days), in March 1944 from sulfathiazole (6 g in 2 days). On these three occasions 2 to 4 weeks were required for the healing of the skin lesions with compress and ointment treatment. In 1945 there were similar skin symptoms from sulfapyridine (12 g in 2 days) and the patient entered our care. He was now treated with vitamin C (500 mg intravenously daily for 3 days). The skin lesions were healed *ad integrum* in 7 days. In 1946 he had tonsillitis and was given 2.5 g of sulfanilamide for 1 day, with simultaneous injections of vitamin C (»Cantan» Bayer), at first intravenously 500 mg per day for 3 days and then intramuscularly 200 mg per day for 10 days. No skin lesions or nausea whatsoever occurred in this instance. — At these times the vitiligo spread to the sites of manifestation of the SA hypersensitivity.

In 1941 the patient, as far as he knew, used saccharin for the first time. Following the ingestion of the food sweetened with saccharin he felt nausea and 5 minutes later had an attack of vomiting and slightly later diarrhea. This reaction recurred each time he took food or drink

TABLE 1

RESULTS OF PATCH TESTS (READINGS AFTER 12 HOURS)

1. Benzolsulfonic acid (sodium salt)	±
2. p-Toluol sulfonamide	±0
3. Dichloramine T = p-Toluene sulfonedichloramide	+
4. Phenacetin (acetylated phenetidine)	−
5. Anaesthesin (p-aminobenzoic acid ethyl ester)	±
6. Crystallose (sodium salt o-saccharin)	−
7. p-Toluidine	−
8. Benzoic acid	(±)
9. Amidobenzolic acid	±
10. o-Sulfobenzoic acid (ammonium salt)	±
11. Sulfanilic acid	+
12. o-Toluidine	−
13. Toluylsulfonamide	−
14. Chloramine-T	+
15. Solvent control (ethyl alcohol)	−
16. p-Aminobenzoic acid	−
17. Sulfanilamide (p-Aminobenzenesulfonamide)	No local redness, but an intense itching on the cheeks at the sites of erythema exsudativum multiforme

containing saccharin, but if the stomach was rapidly emptied by vomiting there was no diarrhea. When taking saccharin he always at first felt a sweetish sensation of taste, which was followed immediately by a bad, undefinable taste. — Another sweetening substance frequently used by the patient, i.e. *dulcin* [1], produced no gastro-intestinal symptoms but merely an unpleasant taste similar to that in the case of saccharin.

[1] »Dulcin» or »Sucrol»: (4-ethoxyphenyl) urea, p-phenethylcarbamides, p-phenetolcarbamide. ($C_2H_5O-C_6H_4-NH \cdot CO \cdot NH_2$).

This case can be briefly characterized as follows:

1) The patient was hypersensitive to SA preparations, manifested by skin lesions as described above. The SA hypersensitivity is probably to be regarded as allergic, as the skin reaction occurred only after repeated use of SA drugs, which produced the sensitization, but after the first occurrence they recurred regularly very soon after ingestion of even small doses of SA drugs.

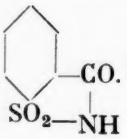
2) The SA allergy was of a group reaction type, as at least three different preparations produced the symptoms.

3) In addition to the allergy for SA there was also hypersensitivity to saccharin, which was manifested by intestinal symptoms (vomiting and diarrhea).

4) The symptoms of hypersensitiveness to saccharine appeared when saccharine was used for the first time but before the appearance of SA allergy.

5) This is presumably the first reported case of saccharin allergy. The observations supplement in a way those of Rogers (5) and Sonck (6), which pointed to an allergic group reaction beyond the field of SA preparations covering also local anesthetics resembling these preparations in some respects, (i.e. in regard to the p-aminobenzoic acid grouping.)

This patient was evidently allergic to SA and saccharin. It is also probable that the reaction against sulfonamides was dominant and accordingly that it occurred as an allergy to the configuration,

although it was manifested only as intestinal symptoms in the case of the saccharin configuration 

The two configuration are closely related.

In order to study this reaction, patch tests with 19 related compounds were performed. The results of these tests are shown in table 1. As will be seen, the results were negative in the skin tests with saccharin, but this was true also of the tests with SA, i.e. the substances for which the skin had specifically acted as the organ of allergic manifestations. In the skin tests carried out simultaneously with different substances there was itching in some of the sites of the SA allergic skin symptoms. This reaction is probably to be regarded as allergic and provoked by simultaneously performed SA tests.

SUMMARY

The case described is presumably the first report on saccharin allergy in which strong gastro-intestinal irritation, i.e. vomiting and diarrhea, was seen immediately after each ingestion of food or drink sweetened with saccharin. As, furthermore, a similar reaction was not caused by another sweetening substance, dulcin, which is hardly distinguishable by taste qualities from saccharin, allergy offers the only explanation for the phenomenon. This interpretation is borne out by the fact that the patient was allergically stigmatized in many ways, and perhaps above all by the fact that he had a polyvalent hypersensitivity to SA drugs.

In view of the similarity in the chemical structure of SA and saccharin it does not seem impossible to explain the intolerance reaction produced by saccharin in the light of a still wider group allergy. The question may mainly be one of polyvalent but specific hypersensitivity, which was not manifested in the patch tests. However, the line from saccharin allergy to polyvalent sulfonamide allergy is fairly evident.

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FROM THE WIHURI RESEARCH INSTITUTE

PHAGOCYTIC ACTIVITIES OF EOSINOPHIL LEUKOCYTES

By

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(Received for publication August 13, 1950)

The knowledge of the phagocytic activities of the eosinophil cells of the blood is, so far, inadequate. The most recent textbooks and manuals of haematology and pathology either state briefly that the phagocytic properties are non-existent in eosinophil cells or record that they are present only in a slight degree. Both Achard & Ramond (1) and Tanabe (9) have found insignificant the phagocytic action of the eosinophil cells of normal blood. Somewhat more attention has been directed to the phagocytic activities of eosinophil cells in connection with eosinophilia. Bushnell & Williams (3), for instance, report that in a case of dermatitis herpetiformis, with eosinophilia 77.2%, the phagocytosis of the eosinophil cells was poorer than in normal blood. Jacobstahl (7) states that in eosinophilia associated with Hodgkin's disease, both staphylococcus aureus and micrococcus tetragenes, and moreover, the tubercle bacillus quite particularly, are phagocytosed readily by eosinophil cells. On the basis of two cases of eosinophilia, in one of which this condition was produced by periarteritis nodosa and in the other by an unknown agent, Herzog (6) held that both staphylococcus aureus and streptococcus viridans are phagocytosed by eosinophil cells in a marked degree, but considerably less readily, however, than by neutrophil leukocytes.

The phagocytic action of eosinophil cells is found to be null in pus (2, 4, 5), whereas this action is claimed to be considerable in the eosinophil cells of pleural fluid (1, 8).

The disagreement of the reports on the phagocytic activities of the eosinophil cells in eosinophilia may be attributed to technique employed. The observations have been restricted to demonstration of those bacteria which are phagocytosed by leukocytes in the eosinophilic condition (e.g. Bushnell and Williams) or to an isolated determination of the phagocytic action of the eosinophil cells in eosinophilia, without securing a control material by determining the phagocytosing power of the eosinophil cells of normal blood under the same conditions. As, in our opinion, the reports on the phagocytic activities of the eosinophil cells in normal blood and in eosinophilia are inadequate and even incompatible, we have felt calling attention to this question justified.

An examination of the phagocytic action of the eosinophil cells has been carried out as follows: 5 cc of venous blood was withdrawn for the purpose of study. The blood was defibrinated with glass beads. The separation of the serum was effected by centrifuging the blood for 10 minutes at the rate of 500 revolutions per minute, whereat the leukocytes accumulated to form a thin layer on the borderline of the serum and the red corpuscles. One cc of this layer was pipetted off into a small sterile test-tube. The staphylococcus aureus suspension employed was prepared from a 24-hour-culture in normal saline. Its bacterial content was from 1500 to 2000 million bacteria per 1 cc as estimated according to the degree of turbidity of the suspension. 0.1 cc of the bacterial suspension was added to the sample of leukocytes to be tested, which mixture was then shaken thoroughly and incubated at 37° C for 10 minutes, being kept in motion all the time. The occurrence of phagocytosis was detected in the following manner: From each tube a number of samples were taken, spread over a microscopic slide in the routine way, and stained partly by May-Grünwald-Giemsa's method and partly by Hansel's, whereby both the leukocytes and the bacteria become stained. The phagocytic properties of the different leukocytes were assessed by Leishmann's method, by counting 50 cells of each type and estimating their bacterial content. As the number of bacteria per leukocyte is not always equal in these tests, the findings on the phagocytic properties of the eosinophils were confirmed by comparing them with the phagocytic action shown simultaneously by the neutrophils.

The material consisted of 3 cases of eosinophilia, which gave the figures of 13.5, 30, and 47%, respectively, and the clinical diagnoses of bronchial asthma (case 1), Besnier's prurigo (case 2), and Loeffler's syndrome (case 3). Three clinically healthy individuals served as a control material (cases 4, 5, and 6), the number of their eosinophil cells ranging from 1.0 to 1.5%. The results of the investigation are given in table 1, where the phagocytosis of 50 eosinophil cells and 50 neutrophil cells is classified according to the number of bacteria phagocytosed. The table also shows both the total number of the bacteria phagocytosed by each cell type and the ratio of the phagocytic action of the two types.

Table 1 indicates that in eosinophilia the phagocytic processes both in the eosinophil and the neutrophil cells outnumber those of the control material. This may partly be due to an unequal ratio of bacteria and leukocytes in the various test series. The increase of the total number of bacteria (335%) phagocytosed by the eosinophil cells exceeds considerably that of the neutrophil cells (48%).

TABLE 1

PHAGOCYTIC ACTION OF EOSINOPHIL AND NEUTROPHIL LEUKOCYTES

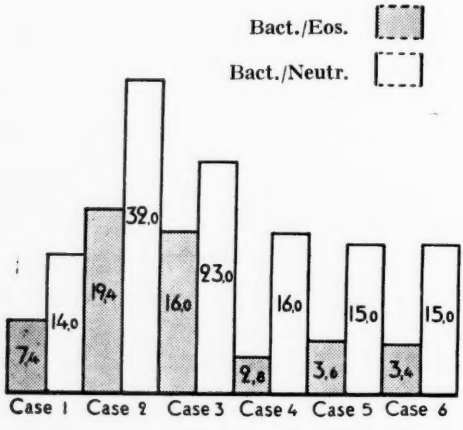
Bact./Leuk.	Cases of Eosinophilia						Control Cases					
	Case 1		Case 2		Case 3		Case 4		Case 5		Case 6	
	Eos.	Neutr.	Eos.	Neutr.	Eos.	Neutr.	Eos.	Neutr.	Eos.	Neutr.	Eos.	Neutr.
0	4	1	0	0	0	0	14	0	9	0	8	0
1–4	9	6	0	0	1	0	24	0	23	1	30	1
5–9	14	8	7	0	8	4	11	4	16	9	11	5
10–14	15	10	8	1	13	7	1	18	1	16	0	15
15–19	5	11	7	3	7	8	0	14	1	10	1	19
20–24	3	11	14	2	14	11	0	14	0	9	0	9
25–29	0	3	4	14	4	5	0	0	0	5	0	0
30–34	0	0	4	11	3	15	0	0	0	0	0	1
35–39	0	0	3	12	0	0	0	0	0	0	0	0
40–44	0	0	3	7	0	0	0	0	0	0	0	0
Total number of phagocyt. bacteria	377	701	970	1605	804	1148	140	821	182	751	172	753
Bact. phag. by eos./Bact. phag. by neutr.	1: 1,9		1: 1,9		1: 1,7		1: 5,8		1: 4,1		1: 4,4	

In the control material the ability of the neutrophils to act as phagocytes is about 5 times higher than that of the eosinophils, whereas in eosinophilia this ability is only 1.6 times greater. It is, in addition, of interest to note that in the 3 cases of eosinophilia the phagocytosing power of the eosinophil cells increase with the progress of the eosinophilia.

Table 2 illustrates the average number of bacteria as phagocytosed by a single eosinophil and a single neutrophil cell in each of the cases.

TABLE 2

AVERAGE NUMBER OF PHAGOCYTOSED BACTERIA PER EOSINOPHIL AND NEUTROPHIL CELL



It appears from what is said above that the eosinophil cells of the blood act as fagocytes, but appreciably less readily than the neutrophil leukocytes. In eosinophilia the phagocytic action of the eosinophil cells show a marked increase.

SUMMARY

The writers have investigated the phagocytic activities of the eosinophil cells both in healthy individuals and in eosinophilia. In three healthy subjects the eosinophil leukocytes were found to act as phagocytes, but the phagocytosing power shown by the neutrophil leukocytes under the same conditions was about five times

higher. In three cases of eosinophilia (the percentages of the eosinophil cells being 13.5. 30, and 47, the clinical diagnoses, again, bronchial asthma, Besnier's prurigo, and Loeffler's syndrome, respectively) the phagocytic action of the eosinophil cells appeared to show a marked increase, whereas even in eosinophilia the neutrophil leukocytes phagocytosed organisms about 1.6 times more readily than the eosinophil leukocytes.

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FROM THE WOMEN'S CLINIC, UNIVERSITY OF HELSINKI

THE EFFECT OF NICOTINE ON THE FEMALE RABBIT AND DEVELOPING FOETUS

AN EXPERIMENTAL STUDY

By

PAAVO VARA and OLAVI KINNUNEN

(Received for publication October 24, 1950)

The question of the toxic effect of nicotine on the organism has been dealt with in a number of even extensive investigations, using both clinical material and animal experiments. Special attention has been paid in recent years to the effect of tobacco smoking on women, especially during pregnancy, not entirely ignoring the ethical aspect of the matter either. Attempts have also been made to investigate the effect of nicotine on the course of pregnancy with animal experiments. Likewise, the relation between nicotine and certain hormonal phenomena has been investigated.

As regards smoking during pregnancy, the extensive investigations of Bernhard (2, 3, 4) seem to show that in a pregnant woman the toxic symptoms caused by nicotine manifest themselves more easily than usual, and would appear especially intensive in the neuro-vegetative field, among others. Thus according to Bernhard hyperemesis appears in smokers in 62.5% (in non-smokers correspondingly in 21.4%), ptyalism in 16.1% (1%), and vegetative disorders of the urinary system, bile and heart in 26.8, 21.4, and 35.7% (13.5, 14.5 and 10.1%). The frequency of occurrence of the pregnancy toxicoses proper was also considerably larger in his material than in the control material: oedema 56.2%

(12.9%), nephropathy 11.6% (3.7%), eclampsism 9.8% (3.4%) and eclampsia 3.6% (0.2%). Further, in his material abortions occurred in smokers in 22.5% and in controls in 7.4%, and earliness in 3.1% and 0.86%; stillbirths were correspondingly 5.1% and 2.3%. Not only during pregnancy, but also in the nursing period, smoking can prove harmful to the child (8, 12, 14, 16, 20).

It thus seems that smoking has an obviously harmful effect both during pregnancy and the nursing period. Many investigations have also shown that tobacco, either by smoking or by transference into the organism in the form of a dust with the air breathed (tobacco workers), causes delay in the menarch age and cycle disturbances and sterility (10, 15).

Experiments to investigate the toxicity of nicotine as regards the ovaries and testes have been carried out for many years. These have been performed either in a smoke chamber or by subcutaneous injections of nicotine in varying doses into the experimental animals. In a number of the earlier investigations, some sort of fibrous degeneration was found in the ovaries, as well as reduction in the number of mature follicles, and cycle disturbances (5, 6, 7, 13, 17, 18, 19, 21). Behrend, Allen and Thienes (1) first drew attention to the difference between the two sexes in the endurance capacity and the lethal dose. On the basis of this observation, Lee (11) performed a series of investigations, using side by side with nicotine a number of sex hormones. His results showed that the male is more easily harmed than the female, and that the corpus luteum hormone forms some kind of protection against the effect of nicotine. Winkler and Fenner (22), etc., later on came to the same conclusion. Grumbrecht and Loeser (9) investigated the effect of nicotine on the reproduction capacity of rats. If the reproduction capacity disappeared, it was due to damage to spermatogenesis. On the other hand, pregnancy in progress was not affected. In the second and third generation, degenerate individuals often occurred.

In addition to the fact that the lethal dose varies in the same species according to sex, the effect on the sex glands is also very different. So far investigations have shown that the changes in the ovary are relatively slight, whereas in spermatogenesis they are conspicuous. For the explanation of this phenomenon Bernhard

(3) says: »Es hängt dies damit zusammen, dass das Ovar nicht neue Eizellen bildet sondern lediglich die kongenitale Eianlage entwickelt und die Eier zur Reife bringt, der Hoden um Gegensatz dazu dauernd neue Samenzellen bilden muss.» It would thus seem that nicotine may most strongly affect cells undergoing rapid division, such as spermatozoa. Therefore it might also be expected that the effect of nicotine on the foetus, whose tissues also undergo intensive development, would be especially strong.

OWN INVESTIGATIONS

The purpose of the experiments was to investigate the effect of nicotine on the ovaries, uterus, Fallopian tubes and vagina of the female rabbit, as well as on the placenta and foetus in pregnant rabbits.

Our material comprised 14 sexually mature healthy female rabbits, whose weight varied from 2.5 to 3 kg.

METHODS

The rabbits were given daily subcutaneously 5 mg. nicotine per kilo of body weight in physiological saline solution. The duration of the experiment varied from 4 to 12 weeks. Half the does were served during the experiments; in two of these probable interruption of pregnancy took place, three were killed for investigations half way through the period of pregnancy (when pregnancy was assumed to have been interrupted), nd atwo gave birth to living young. One of these litters died when 3 to 4 days old. No microscopic or macroscopic changes were observed either in them or in those living longer and killed for investigations. In the three does which were killed during pregnancy, missed abortion caused by nicotine was observed. In all the does, samples were taken from the ovaries, Fallopian tubes, uterine wall, and vagina. From pregnant does, in addition, samples were taken from the placenta and the foetuses. The thyroid was likewise examined in all the rabbits.

RESULTS

A series of illustrations shows the results of our experiments (magnification × 45, except in figure 6, where it is × 450). The preparations have been chosen so that the effect of nicotine is seen in its most typical form.

I. THE EFFECT ON THE OVARY

The changes taking place in the ovary are seen in the germinal epithelium and in the stromal layer near to it. Deeper in the stroma

Fig. 1

no major changes are found. It is due to this that the primary follicles situated immediately below the germinal epithelium are most strongly influenced by the toxic effect and by the major disturbances in the blood circulation. The effect does not seem, however, to extend everywhere even in the germinal epithelium. In figure 1 simple germinal epithelium is seen, which gradually changes into a thick unspecialized layer of connective tissue. Below the connective tissue some primary follicles are seen.

In figure 2 a corresponding thickening of the germinal epithelium is seen. A part of the germinal epithelium is normal with numerous primary follicles. One big secondary follicle is also seen, in which a lumen is just beginning to be formed. Beneath the connective tissue the primary follicles are completely absent.

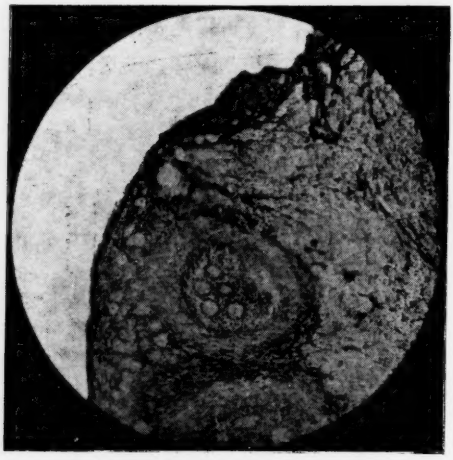

Fig. 2

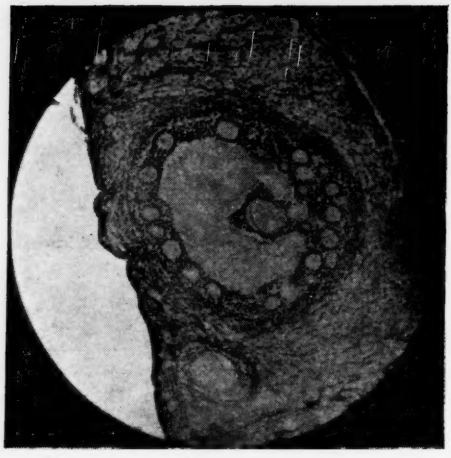

Fig. 3

In figure 3, in the layer of granulosa cells of the developing tertiary follicle, numerous independent small cavities are seen, which have not yet been united with the common lumen.

In addition to becoming a thickened connective tissue, in certain cases the germinal epithelium, with the stroma cells near it, is observed to grow outwards. In this case, a small protuberance furst develops in the germinal epithelium, and gradually becomes

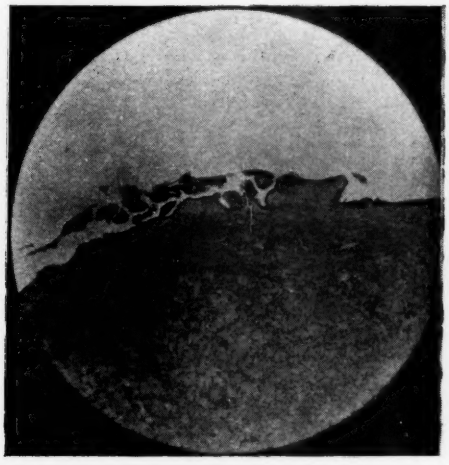

Fig. 4

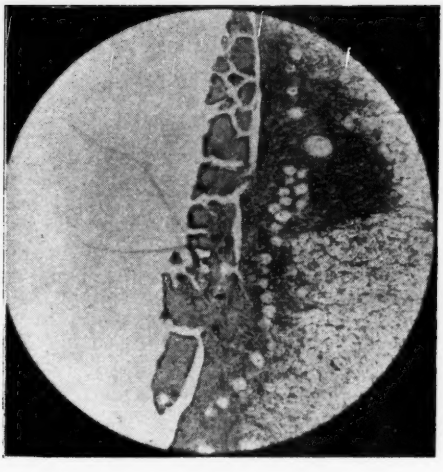

Fig. 5

more thin-walled (fig. 3). On growing further, the protuberance extends and branches, and may even become stalled (figs. 4 and 5). In figure 6 we see the development of a stalk at a higher magnification. At the same time we see the stroma even enter the outgrowth through the stalk.

After prolonged nicotine poisoning the damage to the ovaries is found to be on a larger scale. In the primary follicles the ova and

Fig. 6

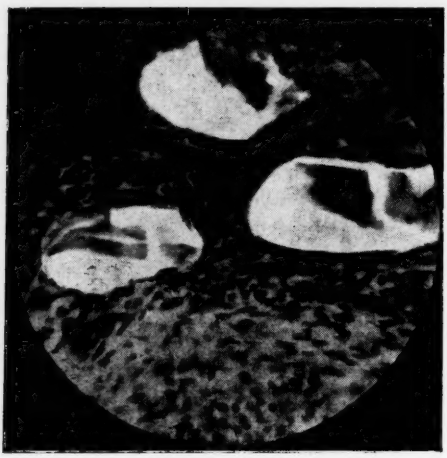

Fig. 7

granulosa cells are seen to have been completely destroyed, the ova having been split into many parts. The view resembles atresia of the ovum. The complete absence of granulosa cells is to be considered typical (fig. 7). The theca of the secondary follicles in thickened and its connective tissue increased; the follicles are compressed. The latter phenomenon may be due to this very increase in the connective tissue (fig. 8).

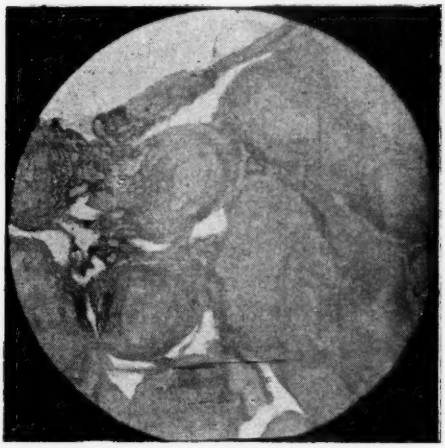

Fig. 8

Fig. 9

II. THE EFFECT ON THE ENDOMETRIUM, PLACENTA, FOETUS AND VAGINA

The secretory phase of the endometrium seems to have remained very active both in the area between foetuses and near the placenta (figs. 9, 10), and may be correlated with the changes in the thyroid to be described later. Likewise, the placenta with its giant cells seems to be well preserved (fig. 11). The tissues of the foetus, on the other hand, are much damaged. The changes are degenerative;

Fig. 10

Fig. 11

the configuration of the different organs has remained unchanged, but the nuclei of the cells are pycnotic. The vagina, on the other hand, is relatively normal (fig. 12).

III. THYROID

The histological structure of the gland shows that the phase in question is very active. The follicles are small and contain little colloid. The epithelium consists of a single layer (fig. 13).

Fig. 12

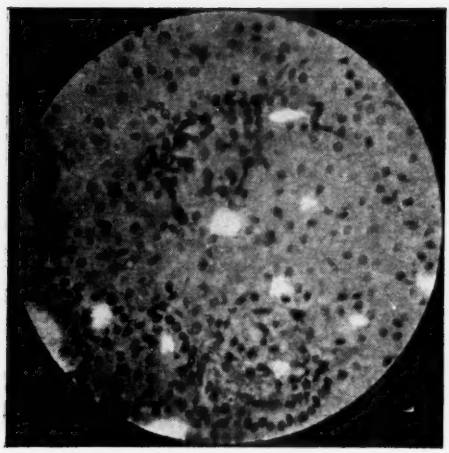

Fig. 13

DISCUSSION

Our results show that the changes in the germinal epithelium of the ovaries are characteristic. On the other hand, in the stroma of the ovaries, the toxic changes caused by nicotine are much smaller. In the endometrium and in the thyroid, strong secretory activity was observed. The placenta tissue seemed to be undisturbed, but the changes observed in the foetus were very strongly histolytic,

the nuclei pycnotic and karyorrhectic. It thus seems that nicotine passes especially readily through the placenta and destroys the tissues of the foetus during the actively dividing phase in the same way as it affects the germinal epithelium of the ovaries.

It seems evident that our observations, indicating that nicotine has a specially strong affinity for developing tissue, are compatible with the previous observations on the destructive effect of nicotine at spermatogenesis. It was interesting to note that all the females served became pregnant in spite of the nicotine treatment. After this the course of pregnancy ran differently in them; the capacity to endure nicotine thus seems to vary considerably even in the same sex. The changes in the thyroid observed by us are also in keeping with the previous observations both on clinical material and in animal experiments.

SUMMARY

The authors describe a number of experiments made with 14 female rabbits, in which during 4—12 weeks 5 mg. nicotine per kilo of body weight was given daily as subcutaneous injections. Half the does were served, and pregnancy began in all these. A probable abortion occurred in two, missed abortion was observed in three, and two produced living young, the litter of one of these mothers, however, soon dying. The toxic effect of nicotine was found above all to influence the germinal epithelium of the ovaries, the primary-follicles, granulosa cells and foetuses. The strongest effect seemed to be manifested in the foetuses, in which great degenerative changes and pyonosis were observed. The endometrium was in an active secretory phase, as was also the thyroid.

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FROM THE WOMEN'S CLINIC OF THE UNIVERSITY OF HELSINKI

THE GROWTH RATE OF EHRLICH'S ADENOCARCINOMA DURING PREGNANCY AND PUERPERIUM

By

VEIKKO KAHANPÄÄ, PAAVO VARA, and SAKARI TIMONEN

(Received for publication October 24, 1950)

The possible effect of pregnancy on the growth rate of carcinoma in man has been discussed extensively in the literature. Döderlein (5), Mayer (11) and Möller (12), for instance, represent the opinion that pregnancy retards the growth of carcinoma. Hinselmann (7), Smith (13), Vignes and Duhail (17) and Wolff (19) even claim that the growth of the carcinoma comes to a complete standstill for the whole period of pregnancy. Others again have maintained that pregnancy does not affect this growth rate, while the most widely held opinion is that carcinoma grows especially rapidly during pregnancy. The opinion represented by practical physicians varies greatly, the most commonly accepted idea being that pregnancy stimulates the growth rate of carcinoma.

Although results of animal experiments are not as such applicable to man, even this path may be considered to lead nearer to the solution of the problem. The results obtained are, however, contradictory. Baatz (1) on the basis of mice experiments has come to some interesting conclusions. According to him the growth rate of carcinoma depends on whether the pregnancy or the cancer began first. *If cancer has not originated prior to pregnancy, pregnancy retards its growth rate. If, however, cancer begins before pregnancy, its growth is increased by this condition.* In either case the growth rate increases during the puerperium.

EXPERIMENTS

In our experiments we have used Ehrlich's adenocarcinoma. The tissue was prepared in sterile conditions, and after macerating in warm saline it was injected into 656 mice. To obtain an exact idea of the growth rate of the carcinoma in non-pregnant animals, it was implanted in such animals only, conception being avoided. Although all the mouse material came from the same firm, representing the same strain, we thought it necessary in our experiments, which lasted one-and-half years, to follow the growth of the carcinoma in non-pregnant mice also. This was done lest the character of the carcinoma might be changed in the course of the continual implantations. In this way the control material has perhaps become unnecassarily large. Those mice which died soon after the implantation (in the course of 10 days) have been disregarded, since the growth of the carcinoma had then only just begun, and it was thus not possible to make any observations on its growth rate. In other animals the growth of the tumour was followed, measurements being made every 4th (—5th) day. In living animals the measurements can only be made in two directions. Consequently only the area, and not the volume, of the tumour was obtained. These measurements obviously provide a satisfactory basis however, for the comparisons of the growth rates in the various experimental series.

MACROSCOPIC OBSERVATIONS

In non-pregnant animals which lived at least 10 days after the implantation the carcinoma grew in 321 cases out of the total of 410, *i.e.* in 78 % of cases. The growth rate, determined as described above, is seen in Fig. 1.

Since pregnancy in mice lasts only ca. 19 days, we have divided the material, in which the mouse was pregnant at implantation, into two groups according to whether the pregnancy had lasted less than 10 days or at least this period. 7—8 days elapse before the tumour is visible after implantation.

Fig. 2 represents the growth rate when the carcinoma was *implanted during the first half of pregnancy.* It is obvious that the tumour has developed more slowly during pregnancy than in the controls. 40 mice were thus treated and 35 of these revealed a deve-

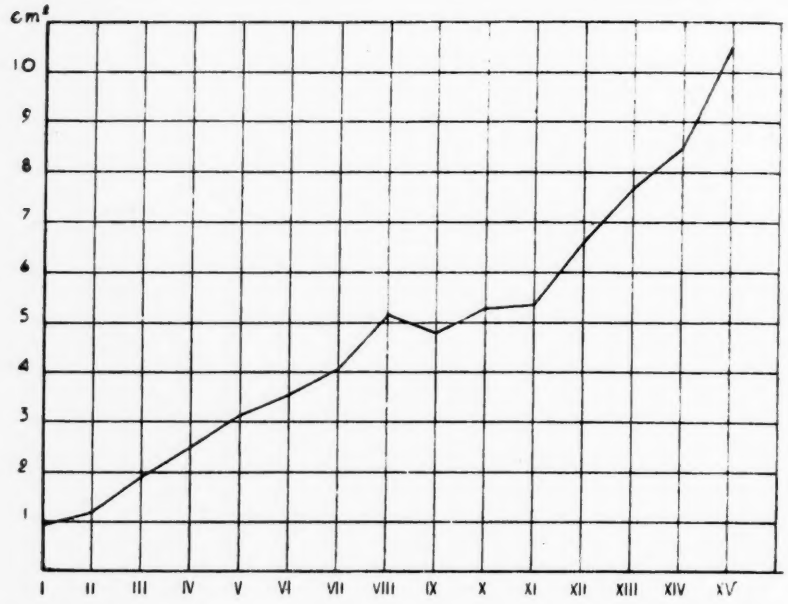

Fig. 1. — Growth of the carcinoma in non-pregnant mice. On the abscissa the times of measurement at 4 (—5) days' intervals. On the ordinate the size of the tumour (cm²).

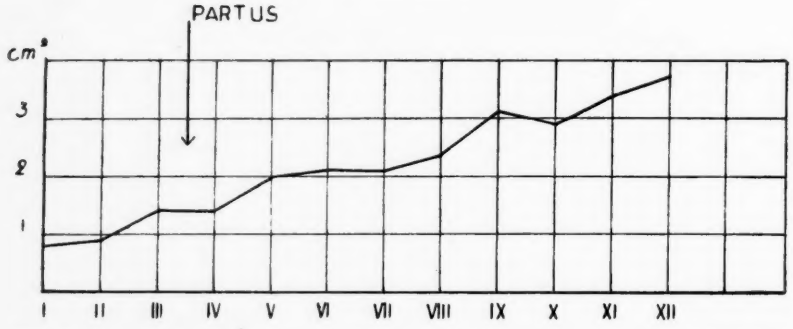

Fig. 2. — Growth of the carcinoma, implantation made during the first half of pregnancy.

lopment of the tumour. No significant difference as compared with the controls was found in the percentage developing growths.

Fig. 3 illustrates the growth rate in the case *when the implantation was made during the latter half of pregnancy*. As the period before parturition during which the tumour could be observed in these cases was very short, Baatz's custom of dividing pregnancy in mice

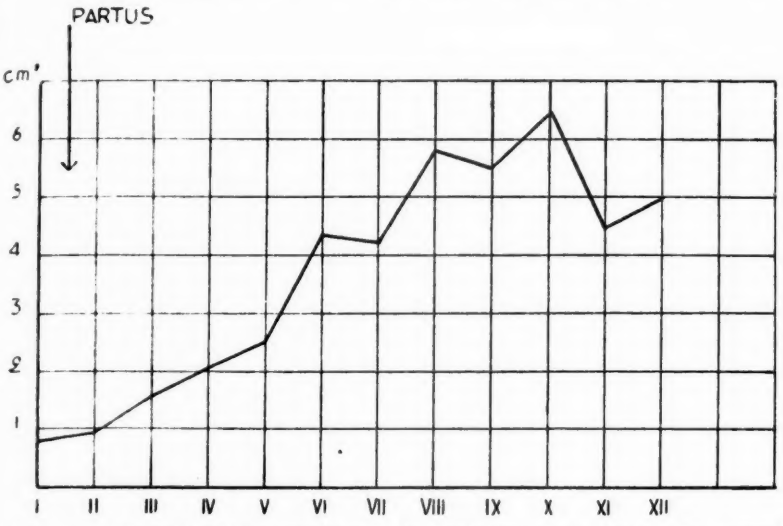

Fig. 3. — Growth of the carcinoma; implantation made during the second half of pregnancy.

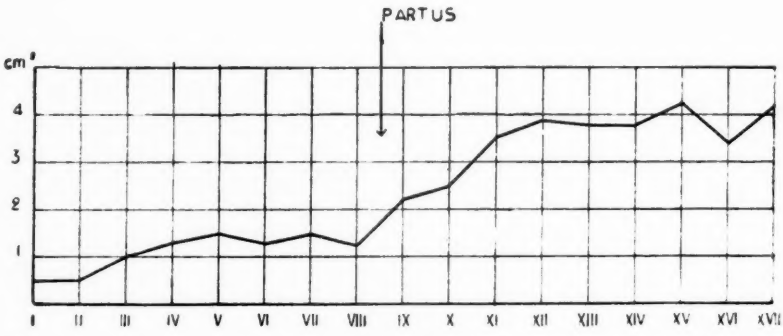

Fig. 4. — Growth of the carcinoma; implantation made before the beginning of pregnancy.

into three periods does not seem appropriate. The beginning of the tumour growth seems to be retarded, as in the previous group. The fact that tumours grew in 19 cases only out of 33 implanted points in the same direction.

Fig. 4 shows the growth rate in cases where *the implantation has already been made before pregnancy*. It is seen that pregnancy constitutes a severe hindrance to the growth of the tumour. Only 46 out of the total of 86 mice implanted developed tumours.

In the puerperium the growth of the tumours in the various expe-

rimental series was always more rapid than during pregnancy (Figs. 2—4). The rate does not, however, exceed that found in non-pregnant animals.

In our opinion the results obtained warrant the following conclusions:

Pregnancy hinders the development of mouse carcinoma, independently of whether the carcinoma has originated before pregnancy or during it. During the puerperium the growth of the carcinoma is stimulated, but does not exceed that observed in non-pregnant animals.

CYTOLOGICAL OBSERVATIONS

The effect of pregnancy on mitosis in cancer cells. It is difficult from the histological picture to obtain an idea of whether pregnancy affects the cancer cells directly, or whether the effect is more indirect. We therefore made observations on mitoses in the carcinoma tissue in order to elucidate the possible presence of intracellular effects. The usual mitotic irregularities (lagging chromosomes, stickiness, and c-effect) have not been found to be confined to cancer (9, 10, 18). Consequently, they could not be used as indicators of the degree of malignancy. Timonen and Therman (14) have established that the most common and characteristic change in female genital cancer is the shortening of prophase, which was found in all the cases studied. For comparison material the normal endometrium was used. In view of this fact Timonen (15) has proposed that the ratio of metaphases and prophases (Prophase Index, PI) might be used to indicate the disturbances in the mitotic timing balance in cancer. In normal tissue this index varies around 1, while the range of variation shown by the different cases of genital cancer was 2—24. In each individual case it was fairly constant, being independent of the frequency of mitoses and the region from which the biopsy was taken. The idea therefore suggested itself that if pregnancy affected the mitoses in cancer cells directly, this effect might be measurable with the aid of the Prophase Index.

Biopsies were taken from 10 mice during all stages of pregnancy and after parturition. In each case the control biopsies were taken from the original tumour. Acetic-alcohol (1: 3) was used as fixing fluid, and after fixation the biopsies were made into Feulgen

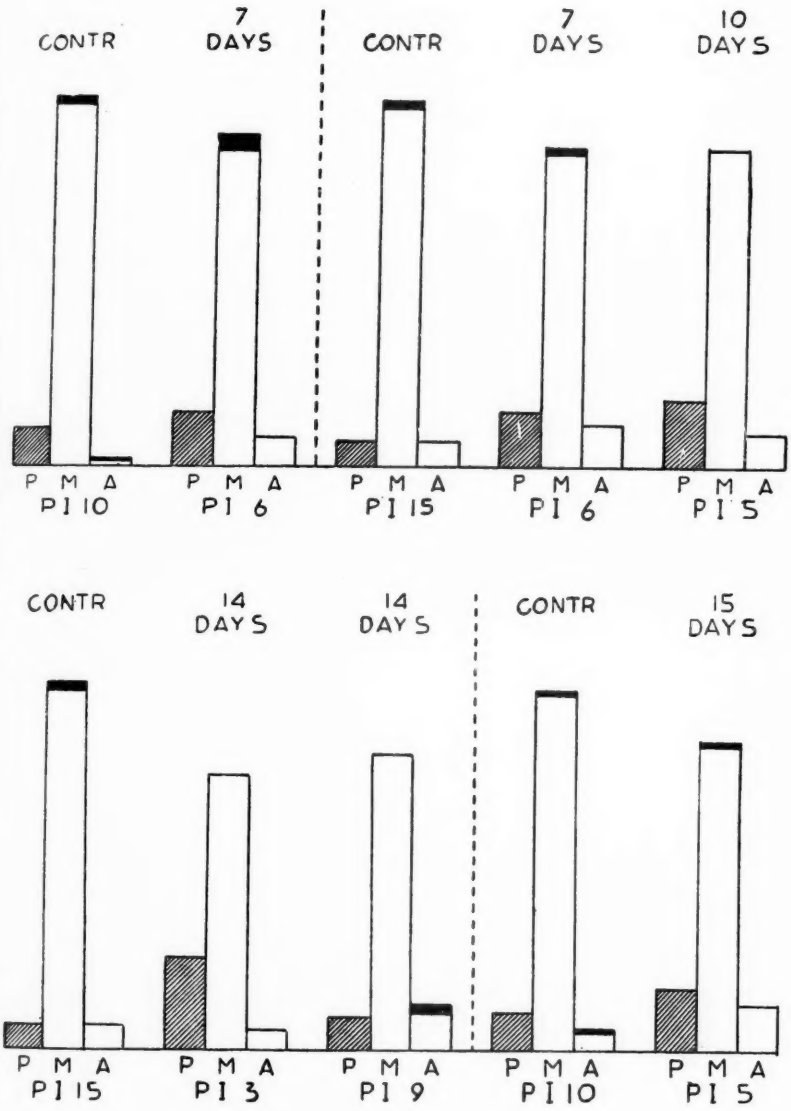

Fig. 5. — The effect of pregnancy on the relative duration of the mitotic phases. Multipolar divisions black. PI-Prophase Index.

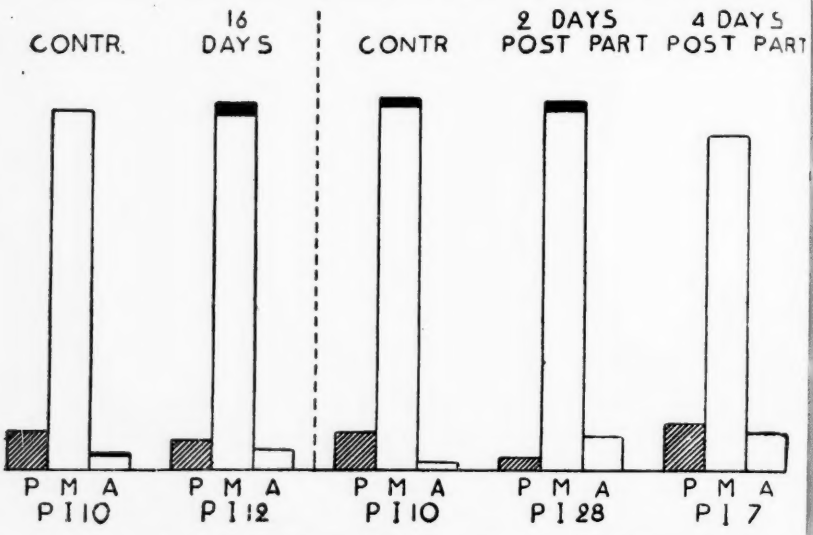

Fig. 6. — The effect of the second half of pregnancy and of the puerperium on the relative duration of the mitotic phases. Multipolar divisions black. PI-Prophase Index.

squash preparations according to Darlington and La Cour (4). The chromocentres in the resting nuclei of Ehrlich's mouse carcinoma are much more distinct than in human cancer cells. With the fixative used they often have the appearance of almost whole chromosomes. Consequently, interphase and early prophase are difficult to distinguish. The count here presented contains therefore late prophases only. Multipolar divisions were also scored, as they are closely connected with the shortening of the prophase.

Fig. 5 shows that the PI in non-pregnant mice is, as a rule, 10—15. During the first half of pregnancy the values decreased — 6, 6 and 5. During the second half the PI was still low —3, 9 and 5, except in one case (Fig. 6) in which it was higher —12. Immediately after delivery the values of the PI increased, to decrease again in a few days reaching values even below the normal — 7. The observations are preliminary and they have not been analyzed in detail. It seems, however, obvious that intracellular changes take place in cancer during pregnancy. The observations suggest that the agent which affects cancer during pregnancy has a direct influence on the cancer tissue itself. According to the precocity theory of the spindle mechanism (14, 15) the

changes observed are best interpreted as a retardation of the extrachromosomal changes in the cell. The decrease in the precocity of the spindle mechanism may be connected with the decreased growth rate of the tumour during pregnancy.

DISCUSSION

Zondek, Zondek and Hartoch (20), who have also found that the growth of carcinoma is retarded during pregnancy, maintain that this effect is caused by prolan. The small experimental series with Ehrlich's adenocarcinoma carried out by Kok (8) showed that pregnancy inhibited tumour development. Kok cautiously interprets his results with the Abderhalden reaction as that pregnancy and carcinoma both give rise to similar phenomena in the organism. Barbanti (2) again claims that the decrease in the tumour growth depends on the changed activity of the ovaries. Fränkel and Geréb (6) have proposed that the decrease in growth, which they found during the latter period of pregnancy and in the puerperium, result from a relative vitamin deficiency. Baatz agrees with the author's, who maintain the decrease in growth rate to depend on placental factors. This »placenta hypothesis» however, is not in accord with the view of Baatz (1) that the growth of the tumour decreases or increases according to whether the pregnancy or the carcinoma is primary.

Leaving aside the question of which agent causes the retardation of the tumour growth, the cytological observations presented above seem to indicate that one of the results is a change in the character of the mitotic mechanism. Caspersson and Santesson (3) have put forward the hypothesis that the origin of cancer may be connected with an intensive nucleic acid metabolism, and especially that increased amounts of nucleic acids would stimulate cell division. Independently of whether or not this much-criticized hypothesis holds true, it is obvious that the increased frequency of cell divisions must be correlated with an increased demand for nucleic acids. If we consider the simultaneous occurrence of carcinoma and pregnancy, it is possible that the developing embryo needs large amounts of substances necessary for nucleic acid synthesis. This in turn would inhibit cell division in cancer tissue. It must also be kept in mind that the nucleic acids are the most energy-rich substances in the animal body.

There are thus several possible interpretations of the inhibited growth of carcinoma during pregnancy, and many more can be advanced. It is, however, obvious that this problem needs further study for its solution.

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FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI

THE EFFECT OF AUREOMYCIN ON E. COLI IN VITRO

By

W. J. KAIPAINEN

(Received for publication September 7, 1950)

The results attained with aureomycin in the treatment of urinary tract infections, caused by *E. coli* have generally been very good (1, 2, 4, 5,). The concentration of aureomycin and the sensitivity of *E. coli* (naturally) are indeed the principal factors on which the result of the treatment is dependent. Although the mode of action of aureomycin *in vitro* is different from that *in vivo* (3), certain aspects that may be of importance to the therapy may emerge from tests carried out *in vitro*. The greatest weakness of tests carried out *in vitro* is the relatively quick loss of aureomycin potency, particularly in neutral and alkaline media at incubation temperature (3, 6, 7). The mode of action of aureomycin being, as known, more bacteriostatic than bactericidal, the bacteriostatic growth limit, with the loss of potency of aureomycin, tends to move closer to the bactericidal growth limit when the incubation period is extended. Although some loss of aureomycin potency takes place in the body, and although some active neutralisation may also occur (3), the bacteriostatic and bactericidal concentration required in the body, however, is considerably lower than in the tests carried out *in vitro*.

PRESENT STUDIES

In studying the *bacteriostatic concentration of aureomycin required against E.coli in the different urines*, a standard *E.coli* strain was used; the medium consisted of albumin- and sugar-free urines from

a single individual and urine mixtures from 10 persons (pH 7). The growth results of the serial two-fold dilutions of aureomycin were read after 24 hours. It was found that the growth limit varied considerably in different urines. This can hardly be ascribed to the technique since growth difference in a single tube was a rare finding in the simultaneously made control series using Fraenkel's solution as a medium.

Since aureomycin has been found to be more active when the solution is acid (3, 6) a study was made of this point using the mixture of 10 persons' urines as a culture medium; it was divided into four portions and the pH's were adjusted to the desired levels (*p*H 5, 6, 7, and 8). Readings of growth results were taken after 24 and 48 hours, Table 1.

TABLE 1

AUREOMYCIN CONCENTRATION (μG/ML) INHIBITING THE GROWTH OF E.COLI IN THE URINE AT DIFFERENT HYDROGEN ION CONCENTRATIONS

Growth Period	*p*H 5	*p*H 6	*p*H 7	*p*H 8
24 hours	7.8	15.6	62.5	125
48 hours	7.8	31.25	62.5	125

The concentration required to inhibit growth was distinctly lower in acid urines than in neutral and alkaline urine. The above-mentioned rapid loss of aureomycin potency at high temperatures and in alkaline media has no doubt contributed to producing such large differences.

It is remarkable that the growth results after 24 and 48 hours were practically identical. When, after 24 hours' growth, samples were taken from the tubes of series that had remained clear and inoculated on an agar dish, the bactericidal concentration in this urine mixture was found to be the same as the bacteriostatic concentration after 48 hours. The bactericidal and bacteriostatic growth limits were also studied with several single-individual urines at *p*H 7. It was found that, in most cases, the bactericidal and the bacteriostatic limit were very close to one another, sometimes even identical, but there were urines in which the bactericidal concentration required was 16 to 64 times the bacteriostatic concentration after an incubation time of 24 hours. In broth a distinct

difference was obtained between the 24- and 48-hour growth and the bactericidal concentration.

Any changes in hydrogen ion concentration during the growth were followed by using bromthymol blue as an indicator. In the tubes that had become turbid due to growth, a shift of *p*H towards alkaline could be observed. This change entered more distinctly only during the second day of growth.

The effect of the specific gravity of the urine was followed by using urine mixtures from ten persons as a medium. Urine mixtures were obtained of which the specific gravities ranged from low to high. However, no distinct differences were found in growth results.

Urine mixtures from ten persons (*p*H 7) were also used in studying *the possible effect of albumin.* Human serum albumin was added to the urine mixture to bring the concentrations to 1, 2, 3, and 4 per mil. The same urine mixture without albumin was employed as control. However, the albumin added to the urine produced no changes in growth limits.

The loss in aureomycin potency possibly caused by the urine was studied by keeping aureomycin urine collected by catheterization (*p*H 7) in an incubator at 37.0°C, and by determining the potency of the aureomycin in this urine after a certain number of hours. Loss in aureomycin potency amounting to 50 per cent was observed after approximately 8 hours. A similar test, with identical results, was made with a urine (*p*H 7) to which a certain amount of standard aureomycin had been added. In the two urines a loss in potency exceeding 75 per cent was found after 24 hours. The temperature is likely to have played a decisive part, as no loss in potency was found in aureomycin urine kept in a refrigerator for the same time. The potency of aureomycin in an aureomycin urine (*p*H 6) collected by catheterization kept in a refrigerator for 8 months, had deteriorated 95 per cent.

The loss in aureomycin potency possibly caused by E.coli was studied by adding to urine collected by a sterile method as much aureomycin as was known to still enable a good growth of *E.coli.* A loopful of *E.coli* culture was inoculated into one tube of this urine. Another tube was kept sterile as a control. The two tubes were placed in an incubator at 37°C. Some of the coli urines was filtered after 4, 8 and 24 hours by a Berkefeld filter. The aureomycin potency in the filtered urine and in the bacterium-free

control urine was determined simultaneously by employing an aureomycin-sensitive *Staphylococcus aureus* strain as a test organism. No differences were observed between the aureomycin concentrations in the control urine and the urine contanimated with *E.coli;* hence, it seemed that *E.coli* was not capable of causing a loss of potency of aureomycin.

SUMMARY

1. The sensitivity of *E.coli* to aureomycin was different in different urines.

2. Aureomycin was more active in acid urine than in neutral and alkaline urine.

3. In certain urines, a rather smal difference between the bactericidal and bacteriostatic concentration of aureomycin was observed.

4. The specific gravity and albumin content of the urine was of no significance.

5. *E.coli* did not cause any remarkable loss in aureomycin potency.

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FROM THE STATE SERUM INSTITUTE, HELSINKI

PREPARATION OF SITOLIPIN

By

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The isolation of Sitolipin from wheat germ powder is effected by approximately the same method as was developed by Pangborn for the isolation of Cardiolipin from beef hearts, though, because of certain slight differences between cardiolipin and sitolipin, the method differs somewhat on some points from the Pangborn Method (4—7).

The wheat germ powder to be used as the starting material must be as fresh as possible, i.e. preferably not older than three days and a maximum of one week old.

15 litres of acetone are added to 10 kg of germ powder, agitating vigorously. The agitation is continued for 1—2 hours, after which the mixture is allowed to chill overnight. It is then agitated carefully and filtered, using a Buchner funnel of some 5 litres and vacuum. The extracted germ powder is again mixed with 15 litres of pure acetone and agitated vigorously for some 3 hours.

After agitation it is filtered as above, and this time to as complete dryness as possible. Methanol is poured onto the germ paste until it covers the entire surface of the paste. It is allowed to chill for some time, after which the paste is suction filtered to as complete dryness as possible by means of a vacuum.

The germ powder paste is poured into 15 litres of approx. 96% methanol. It is agitated vigorously for 3—4 hours and allowed to chill overnight. Then agitated again for approx. half-an-hour, it

is filtered as above. The paste is again extracted using 15 litres of methanol, agitating vigorously for 3—4 hours, then filtering and washing the paste again 2—3 times with methanol. The methanol quantities obtained in the two extractions are combined.

Precipitation of sitolipin from methanol solution is best effected in a high vessel with a capacity of approx. 50 litres. Agitating carefully, 20% $BaCl_2$ solution (200 g $BaCl_2$ to 1 litre of H_2O) is added to the methanol, until a flocculating precipitate ceases to form. A surplus of $BaCl_2$ solution will result in a fine powder precipitation. The consumption of $BaCl_2$ solution is approx. 120 ml, or 3.5 ml per litre. The mixture is chilled for several hours, upon which the precipitate is decanted, and the supernatant is discarded. The Ba precipitate obtained contains Sitolipin.

The precipitate isolated by decantation is then centrifuged. Upon that the precipitate is washed once in acetone and once in methanol. Ether equalling four times the volume of the precipitate is added, and so is 5% Na_2SO_4 solution (50 g Na_2SO_4 to 1 litre of H_2O) in a quantity equalling half the volume of the ether amount used. A suspension is formed of the precipitate by careful agitation, after which it is centrifuged. The liquid layer is poured into a separatory funnel. The remaining precipitate is re-extracted with a mixture of ether, 5% Na_2SO_4 and methanol in the ratio 10:5:1. The extract is centrifuged and the liquid layer poured into a separatory funnel. The aqueous layer is discarded, and the remaining ether layer shaken carefully five times with 5% Na_2SO_4 solution saturated with ether. The ether solution is dried overnight with dry Na_2SO_4 (10 g of Na_2SO_4 to 100 ml of ether solution) at 0°C.

The ether solution is filtered at a low temperature. The clear solution is concentrated in approx. 300 ml and poured, with careful agitation, into an amount of methanol equalling 8 times its volume. The resulting precipitate is allowed to sink, and the supernatant is separated by decantation. The precipitate is centrifuged and washed twice in warm methanol. The methanol solutions are combined.

The methanol solution is concentrated in vacuo so as to remove the majority of the ether. 2% saturated NaCl solution is then added to the methanol solution, which is allowed to chill overnight. After filtering, 20% $BaCl_2$ solution is added to the solution until no more precipitate is formed. It is agitated carefully and

allowed to chill overnight. The precipitate is centrifuged and washed once with methanol and twice with acetone.

To the precipitate, of which the volume is measured by comparing with another centrifuge tube containing an equal amount of water, one and a half volumes of dry ether is added. It is thoroughly agitated and then centrifuged in a closed centrifuge tube. The precipitate is then discarded, and in another centrifuge tube, carefully agitated, double the amount of acetone is gradually added to the ether solution. After centrifuging the precipitate obtained is dissolved in the same quantity of ether as above, and precipitated again with double the amount of acetone. This is repeated until the supernatant remains fairly clear (no turbidity). Usually the precipitation must be effected at least five times. After this the precipitate is dissolved in an equal volume of wet ether. Three times the quantity of acetone is added to the solution obtained, which is centrifuged.

The precipitate obtained is dissolved in approx. 200 ml of wet ether, containing 10 volume% of ethanol. The mixture is agitated in the separatory funnel, carefully shaken, in a third of its volume of half-saturated NaCl solution. This is done twice. Finally the same operation is effected without ethanol until the addition of 5% Na_2SO_4 ceases to indicate the presence of Ba in the aqueous layer. The ether solution is then dried with dry Na_2SO_4, filtered and evaporated until dry in vacuo and in a CO_2 current.

The dry paste is immediately dissolved into absolute ethanol by heating to +57°C and agitating carefully. In between the clear liquid is withdrawn by decantation, and the dissolution is continued with a fresh quantity of ethanol until the residue is no longer tough and paste-like but powdery. At this stage, a fairly considerable greenish residue will result.

The alcohol solution contains approx. 3 mg/ml of Na Sitolipin. (The solution is allowed to stand for a few days at +2—+4°C, when a slight precipitate is formed, which is separated by centrifugation.) The preparation contains approx. 0.05% Nitrogen and approx. 4% phosphorus, and is very slightly yellow in colour, or colourless. Sitolipin at this stage is fully serviceable as syphilis antigen, and its specificity seems to undergo no change with further purification.

CONTINUED PURIFICATION

12.5% $CdCl_2$ solution is gradually added to the ethanol solution containing Na Sitolipin of approx. 3 mg/ml (the solution is nearly saturated) with careful agitation, until no further precipitate is formed. Care must be taken not to add an excess amount of $CdCl_2$ as that would result in Cd Sitolipin being dissolved again. It is allowed to chill overnight, and the precipitate is centrifuged and washed once with absolute ethanol. The volume of the precipitate is accurately measured. An equal amount of dry ether is added and agitated carefully. The solution is centrifuged in a closed centrifuge tube until clear. The precipitate is discarded and double the quantity of acetone is added to the nearly colourless ether solution. It is centrifuged. The precipitate is dissolved again in an equal amount of ether and precipitated using only an equal amount of acetone. The last-mentioned precipitation is repeated. After the second precipitation the solution is clear.

The precipitate is dissolved in a small quantity of wet ether. Three times the quantity of acetone is added and it is centrifuged. The precipitate is dissolved in wet ether. The conversion into Na Salt is effected as previously, except that now no 10% ethanol is used but only half-saturated NaCl solution. Treatment with NaCl solution is continued until the addition of a few drops of 10% sodium carbonate solution ceases to indicate the presence of cadmium in the water phase. If the purification has been carried out carefully, the aqueous solution and ether solution layers can be separated fairly easily and do not form a difficult emulsion.

The ether layer is dried on anhydrous Na_2SO_4 overnight. The ether solution is filtered and concentrated but not until quite dry, leaving a viscous suspension.

Na Sitolipin is dissolved in absolute ethanol, adding ethanol gradually while agitating continuously. This prevents the substance sticking to the sides of the container. The solution is heated to +57°C, stirring at the same time, when the small ether amount will evaporate while the Sitolipin dissolves. At this stage a very small powder-like residue only remains undissolved.

The solution is allowed to stand for some days at +2—+4°C. The clear, colourless solution is separated by decantation from any precipitate that may have formed.

The solubility of the preparation made in this way is approx. 3 mg/ml absolute ethanol. This Na Sitolipin contains 4.0 % phosphorus; its Iodine Number is 115, and nitrogen can no longer be indicated in it.

STANDARDISATION OF SITOLIPIN

The syphilis antigen properties of Sitolipin only emerge in conjunction with cholesterol and lecithin. The sensitivity of the reaction is dependent on the ratio of these three substances.

Cholesterol is available on the market in pure form. — The isolation and standardisation of lecithin (egg lecithin) has been described by Pangborn (1). The preparation is simple and quick.

The final description of the structure of Sitolipin still awaits completion. At the moment we know it to be a compound containing over 50% of unsaturated fatty acids. It is probable that the number of their double bonds determines the Iodine Number of Sitolipin, which is 100—105. For iodine number determinations the Rosenmund-Kuhnhenn-Benham method (3) has been employed, modified as a micro-method. The iodine number of sitolipin preparations must be over 95. Preparations with a lower I.N. are oxidised and will hardly serve their purpose.

Sitolipin contains no nitrogen. Preparations containing over 0.1% nitrogen must be purified further. If the Cd Sitolipin purification stage is carried out, the resulting preparation will have a nitrogen percentage of under 0.01. This, however, results in very great losses. In practice this stage can be omitted, once a preparation is achieved containing less than 0.1% Nitrogen. N-determinations we have carried out by the micro-Kjeldahl method.

Sitolipin contains 4.26 % of phosphorus and Iodine Number 119.5 (8). This and the fact that it contains no nitrogen seems to indicate that in its structure sitolipin is close to cardiolipin, which contains 4.18% of phosphorus. The phosphorus content we have determined by the FiskeSubbarow method (2).

In carrying out dry weight determinations caution must exercised against oxidising the sitolipin, which may lead to considerable errors. The evaporation of alcohol is best carried by passing in CO_2. Nitrogen gas, even if washed with pyrogallol, contains enough oxygen to oxidise the sitolipin.

As starting solution for the antigen we have used a solution

containing 0.2% of sitolipin in absolute ethanol. The sitolipin concentration can be determined either

1 by effecting the dry weight determination. The weighing accuracy must be at least ±0 1 mg, in which case the error in weighing a quantity exceeding 30 mg will be under ±0 3%, or

2 by carrying out the phosphorus determination. When the phosphorus content of »pure» sitolipin (4 26%) is known, the sitolipin quantity can easily be calculated.

The antigen properties of sitolipin remain unchanged at room temperature, in a dark bottle and diffuse light, or in darkness dissolved in absolute ethanol, for over a year. In dry condition sitolipin spoils rapidly.

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FROM THE CHILDREN'S CLINIC, UNIVERSITY OF HELSINKI

ANTIDIURESIS DURING SUCKLING IN LACTATING WOMEN

By

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»Milking or suckling involves more than mere relaxation of the teat sphincter followed by withdrawal of milk under gentle suction. For success, active but inconscious co-operation on the part of the lactating subject is required.» (2) A neuro-hormonal mechanism intervenes in the discharge of milk, known as »let-down» in the cow. Ott and Scott (10) were the first to discover that injections of whole posterior pituitary extract greatly increased the milk flow in a goat over a short period of time. The distinction between the secretion and the ejection of milk was first pointed out by Gaines (3), who believed the latter to be due to a reflex contraction of smooth-muscle fibres in the mammary tissues in response to the suckling or milking stimulus, by means of a nervous reflex. He demonstrated that anaesthesia inhibited the »let-down» in a bitch, but this could be overcome by means of an injection of posterior pituitary extract. He did not, however, postulate a physiological role for the posterior lobe of the hypophysis in the suckling — or milking — process, though he drew attention to the similarity between the effects on the mammary gland of the nursing-stimulus and those of the posterior pituitary extract.

Ely and Petersen (1) advanced the present *neuro-hormonal theory* of the »let-down». They showed that in cows in which the motor nerve fibres to one half of the udder had been severed, the »let-down» could be evoked by oxytocin, or inhibited by fright or injections of adrenaline, in both halves of the udder alike. It would

seem that the ejection of milk depends on a balance between circulating oxytocin and adrenaline. Petersen and Ludwick (12), as well as Peeters, Massart, and Coussens (11), have shown that blood from a cow stimulated to »let-down» her milk will evoke a »let-down» when perfused through an isolated cow's udder.

Thus, it may be assumed that the »let-down» is evoked by the following mechanism: Nerves in the teat are stimulated by suckling or milking; the sensory impulses are carried to the central nervous system and cause a release of a hormone from the posterior pituitary, which hormone by the blood stream reaches the breast and acts upon the contractile tissue of the mammary gland (as to this tissue, see 13, 14). Thus the contents of the alveoli are forced into the larger ducts, where they are available to the sucker or the milker. This neuro-hormonal reflex may become, and often becomes, conditioned, and is elicited by visual and auditory sensations connected with the suckling or milking situation.

Ely and Petersen (1) found that a normal »let-down» could be produced in distracted cows by injections of either the oxytocic or the pressor factor, but the former was clearly more effective. Turner and Cooper (16) found that pitressin preparations were more active than would be anticipated from their oxytocin content; they have suggested that the effect might be due to an unknown third factor, present in both types of posterior pituitary extract. There is still some uncertainty about the exact identity of the active factor or factors involved.

Clinical evidence presented by Robinson (15) and Waller (18) suggests that the »let-down» reflex described above as present in cows and goats also occurs in women. It is well known that when a baby pulls away from a full breast for a moment, the milk may be seen to spray out to a distance of several centimetres, thus demonstrating a positive pressure within the breast. Many women have a subjective sensation in the breast a short time after the beginning of the feeding while the baby is seen to swallow the milk in greater gulps or even choke from too much milk. Milk may also be observed to drip from the breast opposite to that which is being sucked.

Newton and Newton (7) have demonstrated in careful experiments on one woman that the amount of milk available to the baby during a feeding under normal conditions could be greatly

reduced by distractions (pain, anger) which affected the mother but presumably not the baby. This inhibition could be overcome by an intramuscular injection of Pitocin (oxytocic posterior pituitary extract). An injection of Pitocin also produced the same subjective sensations as those produced by applying the baby to the breast. In a more recent work (8) the same authorities found that the incidence of symptoms of »let-down» was significantly higher in successful breast feeders than in unsuccessful breast feeders.

The present writers have attempted to approach the question from a different angle, trying to identify the release of posterior pituitary hormone by its antidiuretic effect. It is known that water diuresis is inhibited by the injection of small amounts of posterior pituitary extracts, notably of the vasopressin fraction. Moreover, strong evidence has been presented suggesting that a release of the antidiuretic hormone controls the secretion of urine under physiological conditions (17). The quantitative proportions of the different fractions of the posterior pituitary hormone, when this is released in the body, are not exactly known, but the existing information suggests that the release of the oxytocic and the antidiuretic factors generally occurs, at least to some extent, together (4). Therefore, the demonstration of antidiuresis during the act of suckling or milking has some interest as an attempt to study the hormonal mechanism of the »let-down».

MATERIAL AND METHOD

The experiments were performed on lactating mothers who volunteered as subjects, during the years 1948—1950. Two slightly different methods of experimentation, A and B, were used. Mothers No. 1—8 were subjected to experiments according to schedule A. Later on, the scheme was modified, and altogether 23 experiments and 23 control experiments were made on mothers No. 9—16 according to the second method. Further details of the distribution of the individual experiments are shown in Table I.

Schedule A: The experiments were performed in the morning, after a preceding fast for at least six hours. The time of the experiment was made to suit the normal nursing hours of the mother. To start with, the mother was asked to empty her bladder. After 15 minutes, this was repeated and the volume of the urine passed

TABLE

SUMMARIZING PRESENTATION OF THE MATERIAL, OF INFORMATION ON THE PRSENCE OR ABSENCE OF SYMPTOMS OF THE LET-DOWN, AND OF THE OCCURRENCE OF AN ANTIDIURESIS (POSITIVE RESULT) IN THE SUCKLING OR MILKING EXPERIMENTS AND CONTROLS

Subject	Age Years	Parity	Weight kg	Presence of subj. Symptoms of let-down		Age of Child Months	Tests			Controls		
				Uterus[1]	Breasts[2]		pos.	neg.	in All	pos.	neg.	in All
Schedule A												
1. S.H.	31	I	75	?	?	2	2	0	2	0	2	2
2. (M.H.)	21	I	?	+	+	½	0	2	2	1	1	2
3. (I.V.)	21	I	?	+	?	½	0	2	2	1	1	2
4. (H.V.)	22	I	64	?	?	8	0	2[4]	2	1	1	2
5. (V.L.)	28	I	56	?	?	11[3]	0	2[5]	2	0	2	2
6. (L.T.)	22	I	60	?	?	2 ½	0	2	2	0	2	2
7. (U.K.)	29	I	60	−	+	3	0	1	1	0	1	1
8. (A.K.)	33	III	58	+	−	1	1	1	2			
Schedule B												
9. (E.R.)	22	I	75	−	+	4	2	1	3	1	2	3
10. (E.B.)	20	II	58	+	+	3	1	1	2	0	2	2
11. (A.V.)	24	I	69	−	+	2 ½	0	3	3	0	3	3
12. (I.H.)	26	I	57	+	+	2 ½	2	1	3	0	3	3
13. (H.M.)	22	I	67	+	+	1	3	0	3	1	2	3
14. (A-L.A.)	24	II	77	−	+	2	3	0	3	0	3	3
15. (R.J.)	26	II	75	+	+	2 ½	2	1	3	1	2	3
16. (A.K.)	28	I	54	+	+	11	0	3	3	0	3	3

[1] Contractions or increase of lochia flow (during the first weeks of puerperium.)

[2] Lactorrhea or feeling of »draught».

[3] Child not more breast-fed, mother only milking.

[4] one of the tests with feeding, one with milking.

[5] Both tests with milking.

The possibility of some suggestive influence when the mothers were questioned about the subjective sensations can not be excluded.

was measured, and a sample of it was taken for chloride determination. Immediately after having voided the urine, the subject was made to drink 1 litre of tepid water. The time of starting and finishing the drinking was noted. After this, the subject was asked to empty her bladder every 15 minutes, starting 15 minutes after the mean of the drinking time. Immediately after the third of these urine samples had been taken, i.e. after 45 minutes had elapsed since the mean time of drinking, the suckling or the milking

of the breast was started. If the onset of the diuresis was delayed, we had to wait a little more, until the excretion of urine was beginning to increase markedly. The act of suckling or milking was performed just as the subject was used to do it. The emptying of the bladder was continued every 15 minutes, until 2 hours had elapsed since the drinking of the water. The volume of each sample of urine was measured, and the concentration of chloride in the urine was determined, by using an electrometric titration (6). The volume of urine is expressed as ml per 15 mins, and the excretion of chloride as g of NaCl per 15 min.

Some of the subjects objected to the great volume of water they had to drink, and a few developed symptoms of water intoxication, for which their results had to be discarded. In addition, it was noted that the subjects were relatively dehydrated at the beginning of the experiment, judging from the volume of urine excreted. Since it is known that an antidiuresis due to the release of posterior pituitary hormone is most easily observed in a hydrated organism (9), the arrangement of the experiments was changed, in a way which made them resemble more the methods of study used by other workers (17) in investigating the posterior pituitary antidiuresis.

This arrangement, schedule B, differed from schedule A in the following respects: A preliminary hydration dose of 5 ml of water per kg of body weight was given 3¼ hours before the actual experiment. The second dose of water was also determined in relation to the weight of the subject, who was now given 10 ml of water per kg of body weight. The breast-feeding in the tests was without exception started at 45 minutes after the drinking of the water, as the babies were schedule-fed, and the feeding was normally due at that hour. In this second series, chloride determinations were omitted.

RESULTS

Secretion of Urine. — A positive result, i.e. antidiuresis, seemed to consist in a drop in the urine excretion curve between 15 and 30 minutes after the beginning of the feeding. To illustrate this, two examples, a »positive» and a »negative» one (case 13: all tests positive, all controls negative; case 11: all tests and controls negative), are given in Figs. 1 and 2, showing the mean curve

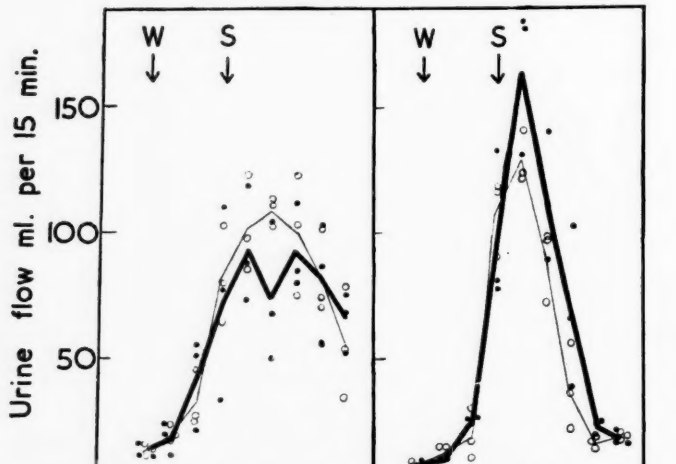

Fig. 1. Fig. 2.

Fig. 1. — Antidiuresis during suckling. Subject No. 13, all tests positive, all controls negative.
Thick line: mean secretion of urine in 3 experiments;
Thin line: mean secretion of urine in 3 controls.
Solid dots: secretion of urine in the individual experiments.
Circles: secretion of urine in the individual controls.
Arrow W: time of drinking the water.
Arrow S: time of starting the suckling.

Fig. 2. — No antidiuresis during suckling. Subject No. 11, all tests and controls negative. The signs as in Fig. 1.

calculated from three tests and three controls, respectively, as well as the volumes of the individual samples.

In some controls, an accidental »drop» of the same kind as in the actual tests was seen, but in group B, the number of positive tests clearly outweighs the number of »positive» controls, as seen in the Table, which presents a summary of the results.

Chloride. — The excretion of chloride varied in the experiments on the subjects No. 1 to 8 between 0.049 and 0.695 g as expressed as NaCl per 15 min. No regularity could be discerned in the behaviour of the chloride excretion as related to the act of suckling or to a consequent antidiuresis. The chloride excretion showed either a rise, a decrease, or no change at all in connection with suckling, independently of whether there was an antidiuresis or not.

A similar observation was made at the corresponding periods of the control experiments. Therefore, the determination of chloride was omitted in further experiments as superfluous.

DISCUSSION

It seems that schedule B was much more appropriate to the present purpose than schedule A. This may have depended on the fact, quoted above, that the antidiuretic effect of the posterior pituitary hormone is not easily demonstrable in subjects who are comparatively dehydrated. Another cause of the failure of schedule A may lie in the fact that the subjects had difficulties in drinking the great amount of water and suffered from nausea afterwards; their resentment might have produced a rise in the adrenaline content of the blood, or it might have acted on the posterior lobe of the hypophysis directly through the central nervous system.

Of course, the possibility cannot be excluded that the tension of the experimental situation may have distorted some results even under schedule B, although the subjects did not report nausea or other discomfort.

On some subjects the tests always gave a negative result. This might depend on there really not occurring a »let-down». From the work of Newton and Newton (8) it would seem that there is a strong relationship between the »let-down» reflex and the ability to breast feeding. At the period when the tests were performed, all the subjects were breast-feeding their respective babies completely or practically completely, or else they had produced a considerable amount of milk over a long period (subjects 5 and 6). They must therefore be classified as a successful breast feeders which, then, would speak against the absence of the »let-down» reflex.

Another explanation would be the existence of a neuro-neural reflex for the »let-down» as presumed in the older theories. At least the experiments on animals, quoted above (1) do not support this theory.

A third possibility would be the release of the oxytocic factor only, without the antidiuretic factor. In order to investigate this, assays ought to be performed of the two separate fractions in the blood or in the urine during suckling. In this connection it may be

appropriate to point out, however, that also the release of the antidiuretic factor can be given a teleological interpretation: The secretion of milk leads to dehydration and, therefore, it is in the interest of the body to conserve water in the anticipation of this loss.

Within the present series, no conclusions can be drawn as to the relationship between the »let-down» reflex and the success of breast feeding.

As a conclusion it may be said that with an appropriate method of investigation it is possible, in a considerable proportion of the cases, to demonstrate an antidiuretic effect of suckling in lactating women. This produces further evidence to support the theory that the posterior pituitary hormone is the active factor in the »let-down» reflex.

SUMMARY

The release of posterior pituitary hormone during the act of breast-feeding was studied on lactating women given a water-load, by means of a determination of the secretion of urine before, during and after a feeding. The subjects were volunteers, all secreting a considerable amount of milk at the period.

With an appropriate method a drop in the urine excretion was often observed between 15 and 30 minutes after the beginning of the feeding. This was interpreted as indicating a release of the posterior pituitary hormone.

There was no regular effect upon the excretion of chloride.

The results are in harmony with the theory of Ely and Petersen (1), according to which the active agent in the »let-down» reflex is the posterior pituitary hormone.

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FROM THE WOMEN'S CLINIC AND FROM THE PARASITOLOGICAL SECTION OF THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI

VAGINITIS PRODUCED BY TRICHOMONAS VAGINALIS

TREATMENT WITH AUREOMYCIN AND TERRAMYCIN

By

RISTO PÄTIÄLÄ and PAAVO VARA

(Received for publication February 16, 1951)

It has long been known that a frequent source of leukorrhea is *Trichomonas vaginalis* inflammation. The vagina does not seem to be the only site for which the protozoon has a predilection. It has also been encountered in specimens obtained by curettage from hypoplastic endometrium, from abortive fetuses, uterine myomata, ovarian cysts, fallopian tubes, placentae, umbilical cords, polypi, and the adipose tissue of the abdominal wall, and in specimens taken in extra-uterine pregnancy and even in appendicitis. Trichomonas is, thus, encountered in almost any part of the genitals and neighbouring organs (13).

From the biogeographical point of view it is interesting that *Trichomonas vaginalis* occurs all over the world (10, 17, 18, etc.).

There are a number of reports on the infestation of children with Trichomonas (2, 6, 7, 10). Ayer and McNeil (1) reported parasitism in a six-year-old girl whose mother was infected with *Trichomonas vaginalis*. Brady and Reid (2) reported a case of vaginal infection transmitted from a woman to her five-week-old daughter. It has been thought possible that newborn infants become infected by their mothers in the course of delivery (15). In order to find whether this assumption was true, Trussell, Wilson, and co-workers (16) examined 41 infants and found that two of them, *i.e.*, 4.8 per

cent, had contracted the infection. It was not possible to follow these cases over a longer period, but the writers thought the view justified that infection with *Trichomonas vaginalis* may be present in small children for years, either manifest or latent. On the other hand, these writers thought, biological factors, such as alterations in the vaginal flora and *p*H, may contribute towards the disappearance of the infection.

Infection, it appears, occurs mainly during the sexual act. In a series consisting of 200 male cases, the urethra was found to be infected in 8 cases (8). It has also been found that infection with *Trichomonas vaginalis* is exceptional among small girls and virgins (10, 15). The infection is commonest at the age of 16—35 years, *i.e.*, when sexual life is most active.

Other means of conveyance by which the infection is transmitted are, according to Trussell, lavatories, swimming-pools, lakes, rivers, and baths.

In Finland, 296 patients were examined for gonorrhea (14). Of these, 150 (50.6 per cent) were found to be infected with *Trichomonas vaginalis*. Part of the cases in the series were also treated for syphilis. Arsenic therapy did not seem to have any effect on the Trichomonas.

Greenblatt found Trichomonas infection in 66 per cent of 127 prostitutes (4). In another group of 378 prostitutes he found 51 per cent of the patients to be infected with the protozoon.

Poor hygienic conditions and promiscuity appear to provide favourable ground for the spread of Trichomonas infection. It is evident that trichomoniasis has to be taken as a borderline case between gynecology and venerology. In view of the principal way of transmission and of the fact that men are both receivers and transmitters of the infection, it will be safest to regard trichomoniasis as a venereal disease with remarkable points of contact with gynecology.

In the series reported by Rouhunkoski (14) (see above), 16 cases were treated partly by Defluon tablets, partly by silver nitrate insufflation. Nine cases gave a positive Trichomonas finding after treatment.

It has been said (15) that although there are a number of drugs which, *in vitro*, rapidly kill *Trichomonas vaginalis*, yet, in the treatment of genito-urinary trichomoniasis, recourse ought to

be had only to such drugs as, while being effective against the protozoon, are least toxic to the host. Thus, the results of experiments on the effect of various drugs on the Trichomonas can only be taken as vague guides to the effect of these drugs in the vagina. Besides, it has to be borne in mind that in clinical practice drugs have to be used in lower concentrations than *in vitro*.

Considering all this, the present writers have made a special study of the effect of certain antibiotic drugs on *Trichomonas vaginalis*. The drugs used for the study were *aureomycin* and *terramycin*[1].

The present writers are aware of only one report (5) of *in vitro* experiments on the effect of various antibiotics (penicillin, streptomycin, tyrothricin, and aureomycin) on *Trichomonas vaginalis*. It was found that the effect of both penicillin and streptomycin was minimal, while tyrothricin and aureomycin proved to be fairly efficient. McVay and co-workers (9) used aureomycin in 54 cases, with a follow-up period of 2—3 months. The patients received the drug in large doses. In 6 cases there was a recurrence. — No experiments have been carried out with terramycin *in vitro* nor, as far as the present writers are aware, *in vivo*.

The present writers' series consisted of 117 cases of Trichomonas infection, all admitted to the hospital as gynecological cases. In all these cases the protozoon was recognised both in the fresh wet discharge and on culture (11).

In all the cases, a sterile urine specimen was taken. This, of course, is indispensable in trichomoniasis. In 24 cases Trichomonas was recognised in the bladder. It is self-evident that such a focus of the protozoon is a constant source of Trichomonas infection.

The following technique was employed. Both the aureomycin and the terramycin were applied to the vagina, by means of a vaginal speculum, every third day from a 250 mg capsule; the capsule was opened and the vagina powdered with the drug. Aureomycin, it is true, could also be introduced into the vagina in a capsule, while the terramycin capsule was not resolved in the vagina. Neither was the aureomycin capsule resolved in all cases. In order to guarantee equal conditions for each case, the capsules were opened and the vagina powdered with the drug.

[1] The writers are indebted to Pfizer & Co., Ltd., for the terramycin used in their experiments.

In a few cases, the drugs were introduced into the posterior fornices in the form of vaginal globules. The globules contained ol.cacao. Both the powder and globules were applied. Three applications seemed sufficient at first; later, however, the number was raised to eight. In the cases of Trichomonas infection of the bladder, 250 mg of the antibiotic in question dissolved in 50 cc of saline were introduced into the empty bladder three times every third day.

The treatment gave the following results:

	No. of Cases	Recoveries	No Final Recovery
Aureomycin	77	52 (67.5 ± 5.35 %)	25 (32.5 %)
Terramycin	40	29 (72.5 ± 7.06 %)	11 (27.5 %)
Total	117	81 (69.2 ± 4.28 %)	36 (30.8 %)

No statistico-mathematical difference can be found between the results obtained with aureomycin and terramycin (Diff. 72.5% −67.5% = 5 ± 8.92%).

In the cases studied, the possibility of immediate reinfection — possibly from the same source as the original infection — was not taken into consideration. The results were considered as such and the follow-up was carried out locally *in casu*. Each case was followed up; in a number of cases this was done weekly. The minimum follow-up period was three weeks. A longer follow-up period seems not to be desirable as to venereal character of Trichomoniasis, but sexual intercourse is to be forbidden during the follow-up period. The examination and treatment of the male partner is also indispensable. A more strict follow-up, *e.g.* in hospital, may probably lead to fewer recurences. Follow-up in all cases was made both from the fresh discharge and from culture. All cultures were made in the parasitological section (Chief R. Pätiälä) of the Department of Serology and Bacteriology in the University of Helsinki.[1]

The results obtained were relatively good, though the follow-up period was not so long as that reported by McVay and co-workers.

[1] The writers have to thank Drs. Markkanen and Kinnunen for their kindness in placing their cases at the writers' disposal, and for generous assistance.

It seems evident that even such a tenacious parasite as *Trichomonas vaginalis* cannot resist the action of the antibiotic drugs of today.

When the results are assessed, particularly from the standpoint of recurrences, it should not be forgotten that antibiotic drugs, which have proved highly efficient against bacteria, may produce alterations in the physiological flora of the vagina and thus render it more liable to recurrences than do other therapeutic agents. (12) On the other hand, it has also to be borne in mind that in this particular disease the possibility of reinfection may be considerably greater than in other conditions.

SUMMARY

The writers studied the therapeutic effect of aureomycin and terramycin in a series of 117 cases of Trichomonas vaginitis. Treatment gave a positive result in 81 cases (69.2 per cent). Aureomycin therapy gave a positive result in 52 cases (67.5 per cent) out of 77 and terramycin therapy in 29 cases (72.5 per cent) out of 40.

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FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI

EFFECT OF TERRAMYCIN ON THE RESISTANCE OF E. COLI

By

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Earlier tests carried out by the present writer (3) showed that when *E.coli* was grown in a culture medium containing aureomycin, increased resistance to aureomycin was accompanied by increased resistance to chloromycetin, but also by a somewhat increased sensitivity to dihydrostreptomycin. When *E.coli* was grown in broth containing chloromycetin, increased resistance to chloromycetin was accompanied by increased resistance to aureomycin and by slightly increased sensitivity to dihydrostreptomycin. In broth containing dihydrostreptomycin an increased resistance of *E.coli* to dihydrostreptomycin was accompanied by slightly increased sensitivity to aureomycin and chloromycetin. The results seemed to indicate that the mode of action of aureomycin and chloromycetin is somewhat similar and different from that of dihydrostreptomycin.

It seemed therefore, that further investigation were called for to ascertain how the increasing resistance of bacteria to terramycin affects the sensitivity of bacteria to aureomycin, to chloromycetin, and to dihydrostreptomycin.

Method. — With *E.coli* as the test organism, a serial 2-fold dilution of antibiotics was made into broth; the growth results were read off after 22 hours. Bacteria were taken from the first turbid tube for the next series. Sensitivity to other antibiotics was determined after seven transfers.

CHANGES IN SENSITIVITY RESULTING FROM THE CULTIVATION OF E.COLI IN A CULTURE MEDIUM CONTAINING TERRAMYCIN

TABLE 1

SENSITIVIES OF THREE E.COLI STRAINS TO TERRAMYCIN, AUREOMYCIN, CHLOROMYCETIN AND DIHYDROSTREPTOMYCIN AFTER SEVEN TRANSFERS IN BROTH CONTAINING TERRAMYCIN (THE CONCENTRATION INHIBITING VISIBLE GROWTH μG/ML)

	Terramycin		Aureomycin		Chloromycetin		Dihydro-streptomycin	
	Before	After	Before	After	Before	After	Before	After
E. coli strain 1	3.9	3.9	3.9	3.9	3.9	3.9	15.6	3.9
E. coli strain 2	3.9	31.2	1.9	62.5	3.9	62.5	31.2	1.9
E. coli strain 3	0.48	31.2	15.6	62.5	7.8	125	0.24	0.24

The sensitivity *E.coli* strain 1 was fairly stable, but it is remarkable that sensitivity to dihydrostreptomycin increased though sensitivity to terramycin remained unchanged. Resistance to terramycin of strains 2 and 3 increased, and was accompanied by increased resistance to aureomycin and chloromycetin, and by increased sensitivity to dihydrostreptomycin with strain 2, while the sensitivity to dihydrostreptomycin of strain 3 remained constant.

CHANGES IN SENSITIVITY RESULTING FROM THE CULTIVATION OF E.COLI IN A CULTURE MEDIUM CONTAINING AUREOMYCIN

TABLE 2

SENSITIVITIES OF THE THREE E.COLI STRAINS TO AUREOMYCIN AND TERRAMYCIN AFTER SEVEN TRANSFERS IN BROTH THAT CONTAINED AUREOMYCIN

	Aureomycin		Terramycin	
	Before	After	Before	After
E. coli strain 1	3.9	3.9	3.9	3.9
E. coli strain 2	1.9	62.5	3.9	500
E. coli strain 3	15.6	125	0.48	31.2

Strain 1 of *E.coli* was stable in this test as well. The increased resistance to aureomycin of Strains 2 and 3, on the other hand, was accompanied by a considerable increase in resistance to terramycin.

CHANGES IN SENSITIVITY RESULTING FROM THE CULTIVATION OF E.COLI IN A CULTURE MEDIUM CONTAINING CHLOROMYCETIN

TABLE 3

SENSITIVITIES OF THE THREE E.COLI STRAINS TO CHLOROMYCETIN AND TERRAMYCIN AFTER SEVEN TRANSFERS IN BROTH THAT CONTAINED CHLOROMYCETIN

	Chloromycetin		Terramycin	
	Before	After	Before	After
E. coli strain 1	3.9	62.5	3.9	3.9
E. coli strain 2	3.9	250	3.9	31.2
E. coli strain 3	7.8	>500	0.48	3.9

The resistance to chloromycetin of *E.coli* strain 1 increased slightly, but the increase was not accompanied by increased resistance to terramycin. The increased resistance to chloromycetin of strains 2 and 3 was accompanied by a slightly increased resistance to terramycin.

CHANGES IN SENSITIVITY RESULTING FROM THE CULTIVATION OF E.COLI IN A CULTURE MEDIUM CONTAINING DIHYDROSTREPTOMYCIN

TABLE 4

SENSITIVITIES OF THE THREE E.COLI STRAINS TO DIHYDROSTREPTOMYCIN AND TERRAMYCIN AFTER SEVEN TRANSFERS IN BROTH THAT CONTAINED DIHYDROSTREPTOMYCIN

	Dihydrostreptomycin		Terramycin	
	Before	After	Before	After
E. coli strain 1	15.6	>500	3.9	0.24
E. coli strain 2	31.2	500	3.9	0.48
E. coli strain 3	0.24	31.2	0.48	0.48

The increased resistance to dihydrostreptomycin was accompanied by slightly increased sensitivity to terramycin in strains 1 and 2. The resistance of strain 3 to dihydrostreptomycin rose, but the rise was not accompanied by any change in sensitivity to terramycin.

E.coli seems react to terramycin in much the same way as we found it to react to chloromycetin and aureomycin. Terramycin, aureomycin, and chloromycetin form a special group differing from dihydrostreptomycin.

Later on the writer found the observations to be compatible with the results obtained by Heilman (2) in similar experiments with *E.coli* and *A.aerog.* strains. Slightly differing results have been obtained by Gocke *et al.* (1) in experiments using agar plates; they found that resistance to terramycin caused only slight increase in resistance to aureomycin.

SUMMARY

1. The increased resistance of *E.coli* to terramycin was accompanied by increased resistance to aureomycin and chloromycetin, but by increased sensitivity to dihydrostreptomycin.

2. Increased resistance to aureomycin was accompanied by increased resistance to terramycin.

3. Increased resistance to chloromycetin was accompanied by increased resistance to terramycin.

4. Increased resistance to dihydrostreptomycin was accompanied by a tendency towards increased sensitivity to terramycin.

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FROM THE STATE SERUM INSTITUTE, HELSINKI, FINLAND

OF THE SPECIFICITY AND SENSITIVITY OF SITOLIPIN AS A SYPHILIS ANTIGEN

By

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In our previous investigations in which, e.g., we compared the Kahn test with the VDRL test, using as the antigen in the latter Sitolipin isolated by us from wheat germs (2, 4), we were able to ascertain that the VDRL tube test with Sitolipin seemed more sensitive and possibly more specific than the Kahn test (1, 2), thus yielding results on the same lines as the VDRL test effected with Cardiolipin (3).

However, the above observations were based on fairly limited material, for which reason it was necessary to carry out additional investigations, based on more comprehensive material, to obtain a more accurate and reliable idea of the relationship of these tests. To this end we have examined 10,000 sera, effecting for each serum parallel and simultaneous Kahn and VDRL tests. The sera, for the greatest part, were obtained from maternity centres and blood donors.

In our studies the sitolipin antigen has been used in the following mixture: sitolipin 0.02% (dry weight), lecitin 0.15% and cholesterol 0.9%. In our preliminary report we used an antigen mixture of sitolipin, lecitin and cholesterol but at that time we still had no pure sitolipin and lecitin and therefore the amounts of the antigen components used were different.

Of the 10,000 tests studied, those in which one or both of the tests were positive totalled 182, while in 9,818 cases both tests were

negative. The breakdown of the said 182 cases, by the tests employed, is as follows (Table 1): Kahn and VDRL-Sitolipin positive 146 cases, Kahn positive but VDRL negative 7 cases, and Kahn negative but VDRL-Sitolipin positive 29 cases.

Kahn	Sitolipin	No. of Cases
+	+	146
+	−	7
−	+	29
−	−	9818
Total No. of Cases		10000

As is seen from the above, out of the 10,000 cases studied, only 7 would not have been detected had we used the VDRL test alone, whereas in the opposite case there would have been no less than 29 such instances. This finding would be justified only if the above 36 patients really had been infected with syphilis and the reactions were absolutely specific. As the latter assumption by no means holds good, we have instituted follow-up inquiries and checks regarding all the patients for whom the results yielded by the reactions differ from one another, to ascertain the number of cases in which either anamnesis or status would reveal something distinctly indicative of syphilis.

In the follow-up examinations, the findings were as follows: Of the seven cases in which the Kahn was positive and the VDRL-Sitolipin test was negative, one had previously received antiluetic treatment, but in the Autumn of 1950 had given birth to a healthy full-term infant in which WaR, Kahn and Sitolipin tests proved negative. In the remaining six cases nothing indicative of syphilis could be found either in the anamnesis or the status. Out of these six cases, in three, in later check-up examinations, the Kahn test remained positive and Sitolipin negative, while in three of them the Kahn test in a subsequent follow-up examination was negative. Out of these three cases, one had had malaria immediately before the first examination, giving then a positive Kahn test while the Sitolipin was negative. Three months after the disappearance of the malaria symptoms the Kahn test became negative. As is well-known, Kahn yields a positive result in a fairly large number of malaria cases while Cardiolipin generally does not, and hence this

instance may perhaps be indicative of Sitolipin possibly reactin to malaria in the same way as Cardiolipin.

In 22 out of the 29 cases in which Kahn was negative but VDRL-Sitolipin positive, distinct, either clinical or anamnestic, indications of syphilis were present. The breakdown of these cases was: 18 treated symptomatic cases of syphilis, 1 case of congenital syphilis, 1 of lues cerebrospinalis, and 2 cases of aortitis luetica. In the remaining 7 cases nothing indicative of syphilis could be found with certainty, but it may be mentioned that in subsequent check-up examinations of these 7 cases the Sitolipin remained positive and in two cases a slightly positive Kahn was present later on.

In judging the results given here we can say that the VDRL test, with Sitolipin used as the antigen, is more sensitive than the normal Kahn test, without yielding any more unspecific results than Kahn. Among the material investigated, 22 cases of syphilis would not have been detected if the Kahn test alone had been used in studying the samples, whereas only one case would have remained undetected if we had employed the VDRL test alone.

As regards the syphilitic cases in which only the VDRL test, with Sitolipin as the antigen, is positive, it is hardly necessary, considering the remarkable sensitivity of the reaction, to take any therapeutic measures if nothing indicative of active syphilis can be found in the patient. On the other hand, it is possible that in the very cases in which the Sitolipin yields a positive result without any symptoms of active syphilis, the patients may subsequently reveal relapses or symptoms of late syphilis, for which reason it may be advisable, nevertheless, to keep an eye on such patients.

In our opinion Sitolipin can be considered as a serviceable, very sensitive and relatively specific syphilis antigen, particularly as its isolation as a pure antigen requires no complicated special equipment but can be effected in practically any laboratory. Another consideration is that the raw material, wheat germ powder, is cheap, readily available and easy to store, nor does it require any special preliminary treatment but can be used handily as such as a starting material.

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